IMPACT OF FOREST HARVESTING ON SOIL MICROBIAL COMMUNITIES AND NUTRIENT CYCLING IN FOREST SOILS OF THE BOREAL PLAIN, ALBERTA

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By

Holly Michelle Hynes

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ABSTRACT

Canada's Boreal forest covers 35% of the landmass, much of which is managed by the natural resources industry. As the largest exporter of wood products globally, the Canadian forestry industry relies on sustainable productivity of the soil. Microbial communities and bioavailability of nutrients are critical components of the sustainability of continuously harvested lands, thus assessing their response to harvesting was the overarching objective of this study. Microbial community biomass and composition was assessed using phospholipid fatty acid (PLFA) analysis and DNA fingerprinting of the bacterial community and ammonia oxidizing bacteria (AOB). *In situ* nutrient availability and chemical soil parameters were also measured here.

Six cutblocks similar to each other except for their age since harvesting were sampled in the summer of 2009 and 2010 in both the forest floor and mineral Ae horizons of Orthic Gray Luvisols of central Alberta in the Boreal Plain ecozone. Microbial communities of these forest soils were generally resilient and adaptable to harvest disturbance over the first ~20 years post-harvest. Soil moisture content emerged as a strong influence on microbial biomass, potentially interacting with the affect of the harvesting activities. There was a flush of nutrients in the first growing season after clear cutting, followed by a consistent decline over time. The AOB community composition changed in parallel with changes in N availability, suggesting that N bioavailability may be directly linked to AOB community structure.

This research contributes to the knowledge that forest harvesting does not necessarily alter the soil ecosystem in a detrimental way. The microbial community adapted to the relatively minor changes imposed by harvesting, as seen by the shift in community composition yet consistency of the total microbial biomass.

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LIST OF ABBREVIATIONS

Ae	mineral horizon of Orthic Gray Luvisol
AMF	arbuscular mycorrhizal fungi
AOA	ammonia oxidizing archaea
AOB	ammonia oxidizing bacteria
B/F	bacterial to fungal biomass ratio
C	cutblock
C_{total}	total inorganic carbon
C/N	inorganic carbon to inorganic nitrogen ratio
DGGE	denaturing gel gradient electrophoresis
FAME	fatty acid methyl ester
FORWARD	forest watershed and riparian disturbance
GWC	gravimetric water content
HY	harvest year
LFH	litter, fragment, humus material – litter forest horizon
MRPP	multiple response permutation procedure
NMS	non-metric multidimensional scaling
N_{total}	total inorganic nitrogen
PCR	polymerase chain reaction
PLFA	phospholipid fatty acid analysis
PRS™	plant root simulator

1.0 INTRODUCTION

Canada's Boreal forest covers 35% of our landmass, and is the country's largest ecozone. This ecosystem is of great economic value to the forestry industry, as Canada is the largest exporter of wood products worldwide (Natural Resources Canada, 2009). As such, sustainable maintenance of our forest ecosystems is of great importance for both the future of the forest products industry and environmental stewardship in general. To investigate the impacts of forest disturbance on the ecosystem a partner project between industry, academia and governmental bodies called The Forest Watershed and Riparian Disturbance (FORWARD) Project was created. This group conducts research on various components of the forest ecosystem including vegetation, amphibians, stream water and soils to assess changes caused by harvesting and wildfire. Furthermore, FORWARD is developing environmental models to be utilized by foresters so that their harvesting activities can be planned and carried out in a manner that mitigates negative disturbances on the ecosystem, which, ultimately, sustains the livelihood of the industry at large.

Within FORWARD, an important aspect of the research is soil quality and biological processes, which forms the base of the ecosystem and dictate the success, or failure, of tree growth. Tree harvesting has been found to have negative impacts on the soil ecosystem, most notably by compacting the soil and removing nutrients. A poorly understood component of the soil ecosystem is the soil biota, though they are absolutely crucial components of soil quality and productivity. Soil microorganisms are responsible for the decomposition of organic matter, nutrient cycling, creating soil structure and symbiotically assisting plants in nutrient uptake, among other services they provide (Attiwill and Adams, 1993; Brady and Weil, 2002). Advances in microbiology and, specifically, molecular techniques have allowed scientists to begin

investigating the identity and function of soil microorganisms in their natural environments. In this study, they are employed in disturbed forest soils.

The overarching objective of this research endeavor was to investigate the changes occurring in the soil as a direct result of forest harvesting, and in the years afterwards. To assess the impacts of harvesting, research sites were established in six cutblocks in central Alberta on the Boreal Plain. The main focus within the soil ecosystem was the microbial community's response to harvesting, and secondarily, changes to nutrient availability. Two microbiological analytical tools were used to investigate changes in the size and composition of both general and specific groups of the microbial community. Nutrient availability was also measured *in situ*, and connections were made between microbial community structure and changes in bioavailable nutrients.

The structure of this M.Sc. thesis is in paper format, with three separate research chapters addressing different research questions preceded by a literature review (Chap. 2). The first results chapter (Chap. 3) presents an assessment of the direct before vs. after impact of clear cutting on soil microbial communities and nutrient availability. Chapter 4 presents a chronosequence study of six differently aged cutblocks in various stages of succession, addressing the question of how microbial community size and structure changed in the years following the disturbance. Chapter 5 investigates a functional group of bacteria critical to the nitrogen cycle in relation to changing nitrogen availability in harvested soils. Finally, a summary and synthesis chapter (Chap. 6) draws the components of the study together and presents final conclusions of the M.Sc. research.

2.0 LITERATURE REVIEW

2.1 Overview of forest soils

Forest soils are generally characterized by the forest floor horizon (LFH), rich in leaf litter, partially decomposed organic material, and woody material. This horizon results from low rates of decomposition commonly found in northern forest soils, which is a function of the wet, cool climates associated with forest ecozones and acidic, nutrient deficient soils. Litter forest horizons are relatively rich in fungi, which are the predominant decomposers in Boreal forest soils (Brady and Weil, 2002). The carbon to nitrogen ratio (C/N) is relatively high in forest soils, which reflects a large content of woody lignified material. Forest soils are often nitrogen (N) limited such that ammonium (NH_4^+) is the dominant inorganic bioavailable form absorbed by plants (Attiwill and Adams, 1993), occurring before it becomes oxidized to its end product, NO_3^- (the dominant form of inorganic N that is taken up by plants in agricultural soils). This oxidation of NH_4^+ to NO_3^- is often the rate-limiting step in the forest soil N cycle (Laverman et al., 2001), thus it is an important area of study. Other plant macronutrients such as phosphorus (P), potassium (K), and calcium (Ca) are of interest in forest soil nutrient cycling because of low bioavailability or minimal natural inputs, often leading to deficiencies for trees. Nutrient limitations become increasingly important when forests are harvested and a significant portion of the above ground N is removed from the ecosystem.

2.2 Affects of harvesting on forest soils

The impact of clear cutting on forest soils depends largely on the forest management practices being implemented, the soil type, and forest cover (Grigal, 2000). Soils underlying

roads and landing strips are more intensively managed, thus are affected more than those of the cutblock area, which experience relatively fewer passes with large machinery (Grigal, 2000). Regardless of the intensity of the disturbance, the surficial horizons of the soil column are more disturbed than deeper layers (Rab, 1986). It has been predicted that soils subject to disturbance related to forestry may take decades to return to pre-disturbed conditions (Corns, 1988; Froechlich and McNabb, 1984), although the extent of the recovery depends on intensity of the initial disturbance and the depth to which the impacts occurred.

2.2.1 Physical properties

Soil compaction is a common repercussion of harvesting operations, and can alter soil bulk density, structure, pore size distribution, air and water infiltrability, water retention, and hydraulic conductivity (Standish et al., 1988). In fine textured soil like a Luvisol, which is the predominant soil type in this study, the impacts of compaction can be more pronounced (Whitson et al., 2003). These changes transform soils into an environment of reduced water permeability, poor aeration and restricted pore space, thus, compaction has direct and negative implications for tree growth (Froechlich, 1979; Grigal, 2000).

Soil erosion can be another major repercussion of harvesting related activities, both during harvest operations and in subsequent years afterwards. During operations, heavy machinery can scrape off surficial horizons, and facilitate their transport to low-lying landscape positions. This is problematic because the surficial horizons, especially the LFH, are rich in substrate carbon and nutrients necessary for plant growth (Grigal, 2000). Individual site characteristics like soil particle size, aggregate stability, slope length and steepness, vegetation cover, and rainfall characteristics define the extent of erosion to occur in each unique scenario

(Chanasyk et al., 2003). Direct implications for tree growth are that a decrease of nutrient availability as well as a loss of the surficial soil horizon, which is the preferred rooting zone, may hamper tree survival. Indirectly, losses to soil microbial communities may further impact tree survival through the various symbiosis and interactions between tree roots and microorganisms.

Harvesting has also been found to induce warmer, albeit more fluctuating (Ballard, 2000) soil temperatures as a result of the removal of the over story vegetation. Zhou and Sharik (1997) found clear-cut soils to be 2-5 °C warmer than undisturbed soils. The removal of trees can cause soils to be either more or less moist, depending on the scenario. In some cases, the lack of tree evapotranspiration results in more moisture remaining in the soil (Zhou and Sharik, 1997). In other cases the increased moisture due to lack of evapotranspiration is counteracted by increases in evaporation caused by warmer soil temperature, resulting in no net change in soil moisture (Chang et al., 1995).

2.2.2 Nutrient cycling

When a forest is harvested, the microsite conditions that govern nutrient cycling often change. Physically, erosion and leaching rates can increase, which transports labile nutrients out of the surface horizons (Grigal, 2000). Soils often become more moist and warm (Zhou and Sharik, 1997), albeit more extreme in annual fluctuations (Ballard, 2000), which can result in increased decomposition, mineralization and nitrification rates (Chanasyk et al., 2003; Walley et al., 1996). Water-logged soils in low-lying landscape positions, or those subject to puddling and ruts can have increased denitrification rates as well, contributing further to nutrient loss from the system. Furthermore, water-logging can induce coupled nitrification-denitrification to further compliment increased rates of both processes (Chanasyk et al., 2003). Upon removal of trees, a

stark decline in organic matter deposition can ensue due to cessation of litter-fall above ground and root exudates belowground (Jones et al., 2003). Some combination of these microsite changes often result in an initial spike in nutrient availability in harvested forest soils, followed by an overall decline in the subsequent years (Grigal, 2000; Pennock and van Kessel, 1997; Simard et al., 2001). This has been observed in numerous studies of stream water nutrient concentrations within harvested watersheds (Bormann et al., 1968; Brown and Binkley, 1994). In cases where natural inputs of a nutrient are less than the removal rate, the availability will eventually drop to below pre-harvest conditions, and this is often the case with Ca (Silkworth and Grigal, 1982). In the case of nutrients that receive relatively higher quantities of natural inputs, like K, N, or P, it has been suggested that the nutrient status of the soil may recover in anywhere between a few years to decades, depending on the environment (Hollis et al., 1978; Silkworth and Grigal, 1982).

Harvesting disturbance can also alter the range of natural variability of nutrient availability in soils. Pennock and van Kessel (1997) found that post harvest soils have a larger range of soil N than mature mixed wood soils (Figure 2.1). The availability of total soil N remains highly variable in recently cut soils (1 yr post-harvest) as well as intermediate aged soils (5-20 yrs post harvest) at sites in Saskatchewan (Pennock and van Kessel, 1997).

Organic matter deposited both above and below ground is decomposed and transformed to bioavailable nutrients, thus contributes to the nutrient status of a soil. The quality and quantity of organic matter deposited can be drastically altered by clear-cutting; above ground by the removal of the trees and belowground by the cessation of root exudates (Jones et al., 2003).

Post-harvest and in the subsequent years of succession, the tree species composition and age-class distribution of a cutblock will continually change, which can also alter the quality and

quantity of organic inputs (Grigal, 2000; Jones et al., 2003). Nutrient availability of a cutblock often becomes greater as time since harvesting increases (Simard et al., 2001), in part because the maturing vegetation contributes more substrate to the soil ecosystem.

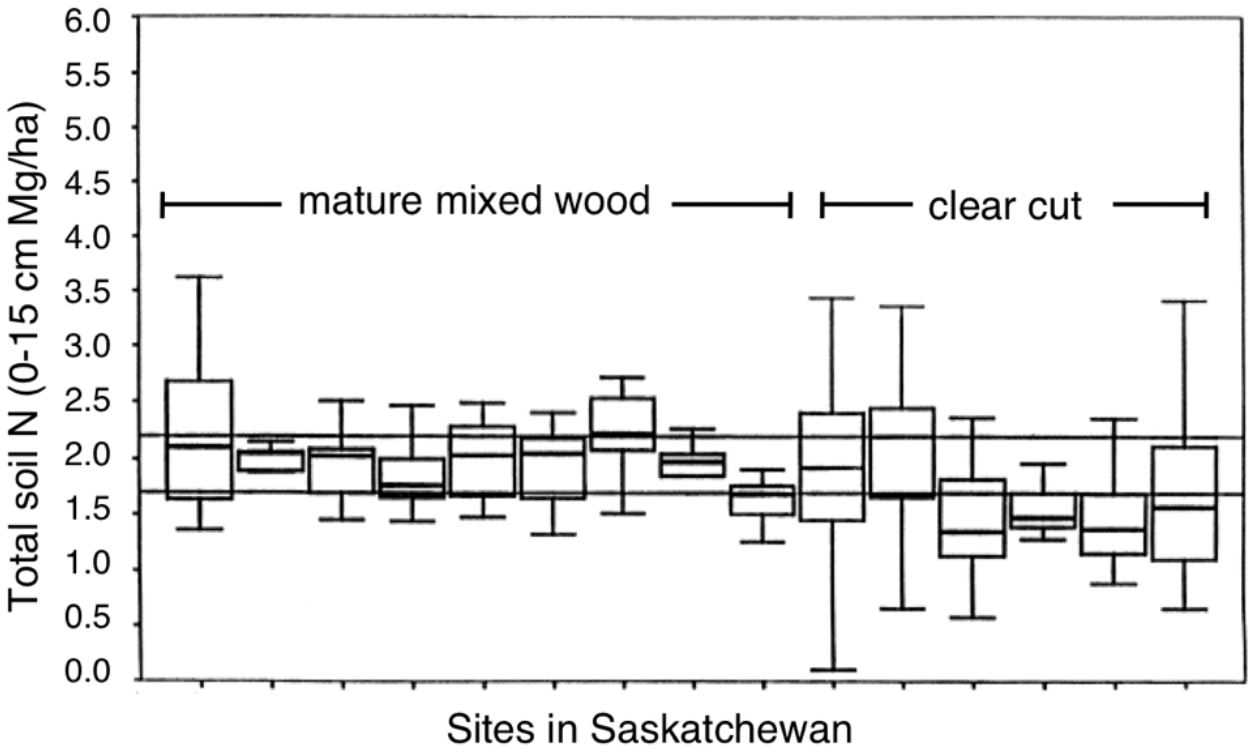

Figure 2.1. Range of natural variability of soil N in the top 15 cm in mature mixed wood forests pre-harvest and post-harvest after clear cutting in a site in Saskatchewan. Adapted from Pennock and van Kessel (1997).

2.2.3 Microbial communities

The effects of harvesting on microbial biomass have been inconsistent in that some studies have found the size of the community to decrease as a result of the disturbance (Bååth, 1980; Bååth et al., 1995; Hassett and Zak, 2005; Pennanen et al., 1999), whereas others have reported no change in biomass (Hannam et al., 2006).

In cases where biomass decreased, the loss was often attributed to a decrease in substrate carbon availability associated with the cessation of litter fall and root additions (Bååth et al., 1995; Hassett and Zak, 2005). A study by Moore-Kucera and Dick (2008) conducted on predominantly Douglas fir (*P. menziesii)* forest soils of Washington state reported that microbial biomass returned to a size comparable to that of an old growth tree stand in somewhere between 8-25 yrs post-harvest (Figure 2.2). In cases where biomass did not decrease, it was postulated that best practices in harvesting operations, such as winter harvesting (Block et al., 2002; Bock and Van Rees, 2002) are successfully mitigating changes in soil microsite, which translates into minimal changes on the microbial community (Hannam et al., 2006; Mariani et al., 2006). In as few as 3 years after harvesting occurred, microbial biomass has been found to have either recovered from the disturbance, or been unaffected by the disturbance in the first place (Mariani et al., 2006; Pennanen et al., 1999).

Fungal biomass is especially sensitive to harvesting disturbance and is often a focus of disturbance studies because it responds more sensitively than bacterial biomass generally does (Bååth et al., 1995; Hassett and Zak, 2005). The decrease in fungal biomass occurs because: a) fungi constitute a large portion of the decomposer community, relying on inputs of organic matter from the trees, and b) mycorrhizal groups of fungi live symbiotically on or within (ecto and arbuscular mycorrhizae, respectively) living plant roots (Bååth, 1980; Bååth et al., 1995; Brady and Weil, 2002; Pennanen et al., 1999). Furthermore, their filamentous nature makes them more reliant on soil structure and pore space distribution (Moore-Kucera and Dick, 2008). For a combination of these reasons and, perhaps others, the fungal contingent of the soil microbial biomass is more sensitive to harvesting disturbance than the bacterial component.

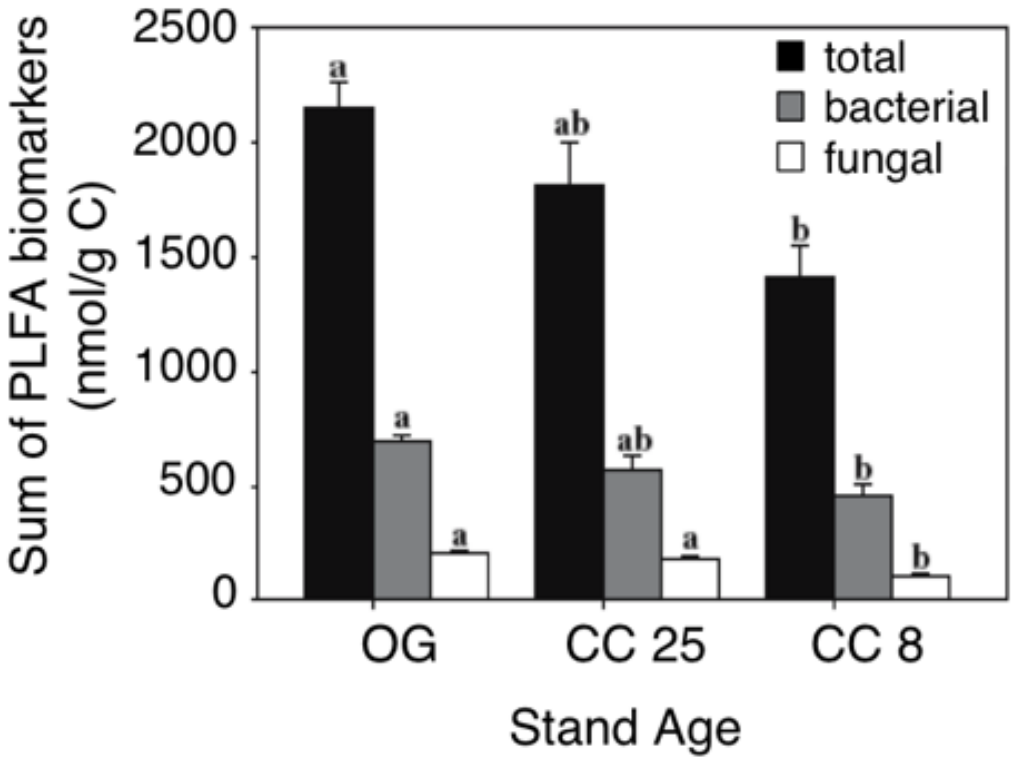

Figure 2.2. Total, bacterial, and fungal PLFA derived biomass (sum of biomarkers) in old growth (OG), 25 yr post-harvest (CC25) and 8 yr post-harvest (CC8) averaged over 7 sampling dates. Letters denote significant differences $p \leq 0.05$ between stand ages. Adapted from Moore-Kucera and Dick (2008).

Bacterial biomass, on the other hand, is less often focused upon in disturbance studies because it is less impacted than fungi (Bååth, 1980; Bååth et al., 1995; Hassett and Zak, 2005; Lundgren, 1982). For example, Ponder and Tadros (2002) found that the effect of organic matter removal and compaction on forest soils did not incur long-term changes on bacterial biomass. Though bacteria are less responsive to disturbance, they are a very important component of the microbial community because they provide many ecosystem services. They are involved in 'virtually all of the organic transactions that characterize a healthy soil system', especially with regard to nutrient cycling (Brady and Weil, 2002). Bacteria mediate the oxidation and reduction

of many macro and micronutrients in the soil, facilitating transformations into and out of bioavailable forms. (Brady and Weil, 2002).

As with microbial biomass, reports of change in microbial community composition associated with harvesting have been inconsistent. Many researchers have found shifts in the community as a result of harvesting (Pennanen et al., 1999; Smith et al., 2008) and attributed the change to increased nitrogen availability (Bäckman et al., 2004), decreases or changes in quantity and quality of carbon inputs (Hendrickson and Robinson, 1984; Moore-Kucera and Dick, 2008), or changes in vegetation cover (Grigal, 2000). The previously mentioned study by Moore-Kucera and Dick (2008) found that the community composition (indicated from phospholipid fatty acid biomarkers) of an 8 yr post-harvest tree stand differed distinctly from 25 yr and old growth stands (Figure 2.3). This study illustrated that by ~25 yrs post harvest the composition of the microbial community is very similar to that of an old growth forest soil. Contrastingly, others have observed microbial community structure to be resilient to compaction (Shestak and Busse, 2005) and harvesting perturbations in general (Hannam et al., 2006; Ponder and Tadros, 2002), implying that best practices can sufficiently minimize soil impacts (Meyer, 1973).

Ammonia oxidizing bacteria (AOB) are a particularly relevant group within the microbial community in relation to harvesting disturbances because they facilitate the rate-limiting step in nitrification (Laverman et al., 2001), which is of special concern in forest soils because they are often N limited in the first place. An enzyme produced by AOB called ammonia monooxygenase catalyzes the oxidation of NH_4^+ to hydroxalamine, and it is this transformation specifically that represents the rate determining step in the N cycle. To date, only one study has been published on the response of AOB to harvesting (Bäckman et al., 2004). They found that the AOB

community shifted in response to clear-cutting, and attributed the change in community composition to NH_4^+ availability, which increased after harvesting. Contrastingly, another study

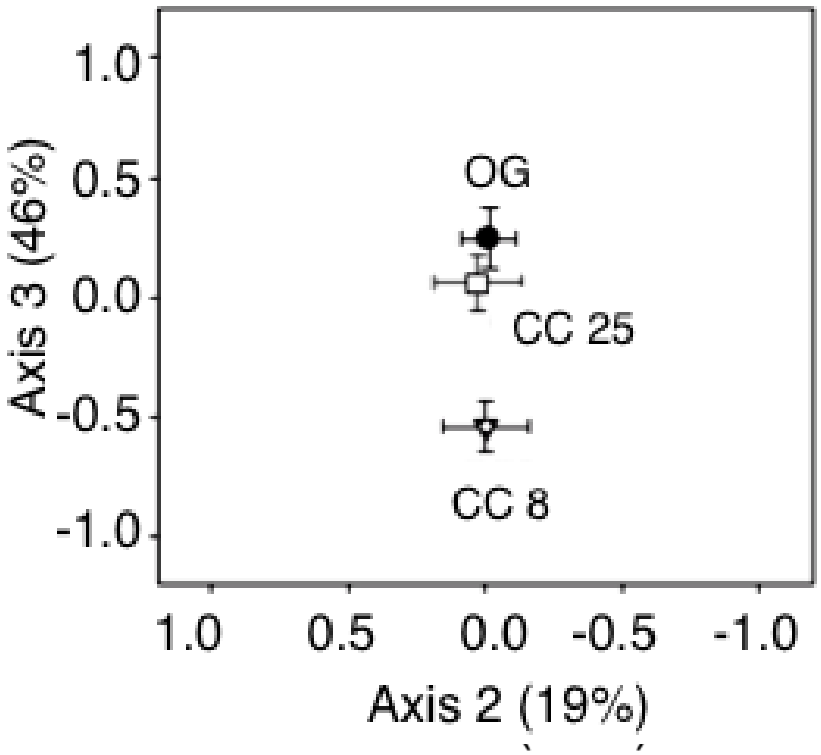

Figure 2.3. Microbial community composition presented in a non-metric multidimensional scaling ordination of PLFA biomarkers in old growth (OG), 25 yr post-harvest (CC25) and 8 yr post-harvest (CC8) averaged over 7 sampling dates. Adapted from Moore-Kucera and Dick (2008).

reported that AOB in forest soils did not change with differing concentrations of ammonium (Avrahami et al., 2002), though this research was not in relation to harvesting.

As illustrated by this review of the relevant literature, there has been some investigation of the changes in microbial communities associated with harvesting; however, the results are inconsistent. Further to the inconsistency in the literature, there is a knowledge gap as to how microbial communities change in size and composition over the course of vegetation succession

because most research focuses only on the first few years post-harvest. There is also limited information on microbial community shifts at a genetic level as most studies utilize more general methods like PLFA or substrate induced respiration. Only one study has investigated AOB response to clear-cutting despite the fact that the activity of these bacteria is of great importance in N limited environments. The research of my M.Sc. thesis was conducted in response to these gaps in the literature with the goal of contributing to our understanding of soil microbiology in the context of the forestry industry, and the need to sustain soil quality and productivity.

3.0 IMPACT OF CLEAR CUTTING ON SOIL MICROBIAL COMMUNITIES AND BIOAVAILABLE NUTRIENTS FROM THE LFH AND AE HORIZON OF BOREAL PLAIN FOREST SOILS

3.1 Preface

Microbial community size and composition are two commonly measured parameters as indicators of environmental perturbations. Microorganisms are useful indicators of soil productivity and health because they are critical components of organic matter decomposition and nutrient turnover. As such, depletion of the microbial biomass or decrease in diversity have been linked to a decrease in soil productivity and quality. In the context of the forest harvesting industry, the use of microbial indicators of disturbance yields inconsistent findings. There is also a lack of studies that have addressed the changes in microbial communities occurring in concert with changes in nutrient availability. Because nutrient availability is known to change as a result of forest harvesting and microbial communities facilitate nutrient turnover and transfer, the connection between microbes and nutrient availability in the context of harvesting is an important issue. Furthermore, there is a lack of awareness of whether soil response to forest harvesting is consistent in differing soil horizons. Thus, the objective of this study was to assess the short-term impact of clear cutting on community level microbial communities and nutrient availability of Boreal Plain forest soils, and to determine whether the LFH and mineral Ae horizons respond the same to the disturbance. The hypothesis was that both microbial biomass and nutrient availability would decline due to harvesting, and that community composition would differ from that of pre-harvest soils.

3.2 Abstract

Soil productivity is primarily governed by the microbial communities inhabiting the soil, and is an important priority within the forestry industry. The objective of this study was to assess community level changes in microbial community size and composition in conjunction with nutrient bioavailability and soil microsite response to clear cutting. Soils from the Boreal Plain of central Alberta were sampled from the LFH and mineral Ae horizons using a time series approach. Phospholipid fatty acid analysis was utilized to determine changes in microbial community size and composition in soils of a cutblock area over three growing seasons spanning pre- and post-harvest conditions. *In situ* nitrogen (N), phosphorus (P), potassium (K) and calcium (Ca) were measured and other chemical soil parameters characterized to compliment the microbial data. Results indicated that there was no loss of microbial biomass until two years after the disturbance, but the community composition was altered in the immediately in both the LFH and Ae horizons. The post-harvest flush of N, P and K reported by several other investigations was observed here in the LFH and Ae, though the response of Ca was inconsistent. Though soil chemical characteristics were relatively undisturbed by clear cutting and the microbial community indicated adaptability by shifting in composition, losses in microbial biomass and nutrient availability may represent challenges to sustainable management of Boreal soils managed by the forestry industry.

3.3 Introduction

Sustaining the productivity of forest ecosystems has become a concern in continuously harvested areas because nutrient availability and soil health have not been maintained (Ponder and Tadros, 2002). Forest harvesting can remove organic material and nutrients from the ecosystem both directly from tree biomass removal and indirectly from post-harvest effects such as erosion and leaching. It is important to understand how harvesting affects the key components of the forest soil ecosystem in order to improve forest management practices to better mitigate soil degradation. The Forest Watershed and Riparian Disturbance (FORWARD) Project is working with forestry companies and governmental agencies to investigate how harvesting and fire related disturbances affect various components of the ecosystem, and to use the data in environmental models to aid forestry companies in developing their management plans in a sustainable manner.

Microbial communities are an important component of the forest ecosystem because they facilitate organic matter decomposition and nutrient cycling in the soil. Thus, their response to harvesting can have direct implications on tree growth (Moore-Kucera and Dick, 2008). Microbial biomass (Bååth, 1980; Bååth et al., 1995; Hassett and Zak, 2005) and community composition (Moore-Kucera and Dick, 2008) have been found to change in response to harvesting, though resilient soil microbial biomass has also been reported (Hannam et al., 2006; Pennanen et al., 1999). Within the total microbial biomass, the saprophytic fungal contingent has been generally found to be more sensitive to disturbance relative to bacteria (Bååth, 1980; Bååth et al., 1995; Hassett and Zak, 2005; Lundgren, 1982). These fungi are highly dependent on lignified substrates derived from mature woody biomass (Hassett and Zak, 2005), and symbiotic relationships with rooting systems. In coniferous forest soils, like those of the current study,

ectomycorrhizal fungi are considered to represent the majority of the total fungal biomass (Finlay and Soderstrom, 1989; Pennanen et al., 1999). Phospholipid fatty acid analysis (PLFA) is a useful method of assessing the viable microbial biomass because the phosphate group is quickly hydrolyzed upon cell death (Zelles, 1999). They are also useful as biomarkers to assess community structure because of their wide structural diversity (Zelles, 1997) and specificity within groups of organisms (Frostegård and Bååth, 1996).

Soil microbial processes produce bioavailable nutrients, which are crucial necessities for plant survival. Long-term managed areas are often limited in macronutrients such as nitrogen (N), phosphorus (P), potassium (K) or calcium (Ca) because repeated harvesting removes nutrients at a faster rate than natural inputs (Silkworth and Grigal, 1982). This is a more common problem in forested environments than agricultural areas because fertilizers are not readily used to replenish the nutrients extracted from the harvested biomass. As a result, macronutrients have generally been observed to decline as a result of harvesting (Hollis et al., 1978; Prichett and Fisher, 1987; Silkworth and Grigal, 1982), though an initial spike of nutrient availability is commonly seen in the first growing season after harvesting (Hollis et al., 1978; Simard et al., 2001).

Very little research has been done to compare the response of different soil horizons to disturbance, despite the fact that it is well understood that the LFH has very different characteristics from the mineral horizons. Both of these horizons are very important for plant growth because plant roots are most densely located at their interface (Strong and La Roi, 1985) and furthermore, top-most horizons are most acutely impacted by harvesting related disturbances (Grigal, 2000). It has been suggested that investigating disturbance by sampling only one soil horizon does not provide accurate interpretations of the whole soil system (Alban, 1982), though

it is very common practice in forestry research to sample one horizon or homogenize some combination of two horizons that fall within the same depth range.

In order to sustain soil health and plant productivity in continuously harvested and replanted forest soils, nutrient loss needs to be better understood and subsequently better managed. An understanding of the microorganisms facilitating organic matter turnover and nutrient cycling and the availability of macronutrients are crucial for the future of the forestry industry. Therefore, the objective of this study was to assess the response of microbial communities and macronutrient availability to clear-cutting using a time series approach. The secondary objective was to compare the response of LFH versus mineral Ae horizons to harvesting disturbance to determine if they depict the same trends as one another. The cutblock area was sampled pre-harvest (2007) and post-harvest (2008-2010) to capture the initial response to the disturbance and the changes occurring in the first three growing seasons afterward.

3.4 Materials and Methods

3.4.1 Site description

The study site was located in central Alberta (54°03' N, 115°84' W; 782 meters above sea level) on the Boreal Plain, 220 km northwest of Edmonton, Alberta. The region is underlain by Cretaceous and Tertiary sandstones, shales, clays and gravels ranging from <15 to >150 m thick (Pawlowicz and Fenton, 1995). The predominant soil type is Orthic Gray Luvisol, which typically exhibits a LFH layer, an Ae horizon leached of clay, and a Bt horizon enriched in clay from the above Ae horizon (Agriculture Canada Expert Committee on Soil Survey 1987). These Luvisols are relatively rich in P and Ca because of high apatite (P) and Ca content of the parent material (Smith et al., 2003). The predominant trees in the area are lodgepole pine (*Pinus contorta*). Other species found in the region are white spruce (*Picea glauca*), balsam poplar *(Populus balsamifera*) trembling aspen (*Populus tremuloides*), jack pine (*Pinus banksiana*), and black spruce (*Picea mariana*) (Smith et al., 2003). The region experiences mean summer and winter temperatures of around 10 °C and -15 °C, respectively, and an average of 500 – 600 mm of precipitation annually (Environment Canada, 2000; Strong and Leggat, 1992).

The site was clear cut harvested in January of 2008. Site preparation consisted of the rake and burn method in which large branches were gathered and burned, while smaller branches were left on-site to decompose. In the first growing season after harvesting the site was treated with Vision (Monsanto Inc.) at a rate of six litres per hectare (active ingredient is glyphosate).

3.4.2 Soil sampling methodology

The cutblock was divided into a systematic sampling plan and sampled as a time series study; samples were taken from the same site in June of 2007 (before harvesting), 2009 and

2010. The site was separated into three transects, each transect with three sample plots, and each sample plot a composite of four randomly chosen subsamples (Figure A.1). Soil was collected using a JMC Backsaver probe (Clements Assoc. Inc, Newton, IA) with a 3.2 cm diameter tip. Samples were gathered from the LFH and mineral Ae horizons, hereafter referred to as Ae, at each sample location for a total of 18 samples per year (i.e., 3 transects x 3 plots x 2 horizons = 18 samples per year). Sampling took place along the slope gradient (if any) to account for any differences associated with slope position. Soils were stored on ice in coolers until returning to the lab, at which point they were sieved with a <2 mm mesh and stored at 4 °C. One set of subsamples was air-dried and pulverized for general soil characterization, while a second set was freeze dried and stored at -20 °C for PLFA analysis.

3.4.3 Characterization of soil properties

All soil analysis were carried out based on the methods described in the Soil Sampling and Methods of Analysis Book (Canadian Society of Soil Science, 2008). Gravimetric water content (GWC) was determined by calculating the mass lost after drying a known quantity of soil at 105 °C for 48 h, and expressed as a percentage of the mass of water per unit mass of dry soil.

$$GWC\ (\%) = 100 \times (M_{swc} - M_{sc}) / (M_{sc} - M_c)$$

Where M_{swc} is the mass of the moist soil and container, M_{sc} is the mass of the dry soil and container, and M_c is the mass of the empty container. Water content of >100% is commonly reported in the LFH horizon or organic soils because the difference between the sample wet weight and dry weight is often larger than the dry weight itself.

Soil pH was measured in a 2:1 soil: double deionized water slurry. Total C (C_{total}) and N (N_{total}) were quantified by the combustion method using a LECO CNS-2000. Organic C (C_{org})

was quantified using a LECO Carbonator model CR-12. C_{total}, N_{total}, and C_{org} were expressed as a percentage (%) of the total soil mass (Helgason et al., 2009). The carbon to nitrogen (C/N) ratio was calculated by dividing the C_{total} by the N_{total} and thus reported here unit-less.

3.4.4 *In situ* bioavailable nutrient measurements

In situ bioavailable nutrient (N, P, K, and Ca) fluxes were measured using Plant Root Simulator (PRS^{TM}) Probes (Western Ag Innovations, Saskatoon, SK) in the summers of 2007 through 2010. The 2008 PRS^{TM} probe data were included from a parallel study within FORWARD (A. Mascahrehnas M.Sc. Thesis, 2011). This technology consists of an ion exchange membrane that emulates the nutrient sorption and surface characteristics of plant roots, thus assesses potential nutrient supply rates. In other words, the flux of nutrient into the PRS^{TM} probe supplies a surrogate measurement of plant available nutrient concentration in soil water, hereafter referred to as bioavailable nutrients. Probes were installed on the same date, and in the same location as soil samples for a total of 18 separate measurements per summer. A 6-week burial time was utilized for the PRS^{TM} probes to ensure saturation would not occur (Western Ag. Innovation, PRS^{TM} probe Operations Manual), thus probes were replaced mid-July of each summer with a fresh probe in the exact same "soil slot". Total burial time was 12 weeks; from early June to mid-August each summer. Upon removal, probes were cleaned with deionized water immediately and stored at 4 °C for subsequent analyses by Western Ag. Inc (Saskatoon, SK). Nutrients were eluted from the PRS^{TM} probes with 0.5M HCl. A segmented flow autoanalyzer III (Bran and Luebbe, Inc., Buffalo, NY) was used to quantify N and P colourimetrically. Phosphorus and Ca were quantified by a PerkinElmer Optima 2000-DV inductively coupled plasma mass spectrometer (PerkinElmer Inc., Shelton, CT).

3.4.5 Phospholipid fatty acid (PLFA) analysis

The method developed by Helgason et al. (2009), based the original protocol of Bligh and Dyer (1959), was used for PLFA analysis. Sieved, lyophilized soil was weighed to 4.0 g and extracted in a methanol/chloroform mixture, then dried down under N_2 flow. Neutral, glyco- and phospho- lipids were separated out using solid phase extraction columns (0.50g Si; Varian Inc. Mississauga, ON), sequentially eluted with chloroform ($CHCl_3$), acetone ($(CH_3)_2CO$) and methanol (MeOH) respectively, and the phospholipid fraction dried under N_2 flow. The phospholipid fraction was then methylated in a solution of 1:1 methanol/toluene and methanolic potassium hydroxide (KOH) at 35°C. The resulting fatty acid methyl esters (FAMEs) were analyzed using a Varian 3900 gas chromatograph with a 50-m Capillary Select FAME column (Varian). Peaks were identified based on comparison to known standards (Supelco Bacterial Acid Methyl Esters and MJS Biolynx). All samples were quantified based on comparison with an internal standard, methyl nonadecanoate (c19:0).

Bacterial biomass was determined as the sum of 10 fatty acid biomarkers, 3OH-12:0, a-12-meth-15:0, i-13-meth-15:0, 15:0, 2OH-14:0, i-14-meth-16:0, i-15-meth-17:0, 10-methyl-17:0ω8c, 17:0, and 2OH-16:0 (Hamel et al., 2006). Saprophytic fungal biomass was represented by the biomarker 18:2ω6c (Frostegård and Bååth, 1996), and arbuscular mycorrhizal fungi by the biomarker 16:1ω5 (Spring et al., 2000). All biomass data reported here represent dry soil weight, and are reported in units of nmol/g soil derived from individual molecular weights of each fatty acid. The ratio of bacterial to fungal biomass (B/F) was calculated by dividing the nmol/g dry soil of bacterial biomass by the saprophytic fungal biomass.

3.4.6 Statistical analysis

Data exploration, correlations and significance testing to compare groups was carried out with SPSS Version 17.0 for Windows (SPSS Inc., 2008). Log-transformed chemical and microbial soil parameters were tested for normality (Shapiro-Wilks) and homogeneity of variance (Levene's Test), and were determined to be non-normal and heteroscedastic, thus statistical tests that do not violate parametric assumptions were used to analyze the data (Field, 2005). The Kruskal-Wallis test, which compares independent groups, was used to analyze the effect of clear cutting and soil horizon on chemical and microbial parameters, and correlation coefficients were derived using Spearman's rank correlations for non-parametric data (alpha was 0.05).

Non-metric multidimensional scaling (NMS) using PCOrd v.5.0 (MjM Software Gleneden Beach, OR) was used to analyze the community composition of the PLFA data (Helgason et al., 2010; Swallow et al., 2009). This method of ordination is appropriate for ecological datasets that are nonparametric (McCune and Grace, 2002). Phospholipid data was expressed as mol% and was transformed using log (mol% + 1) as recommended in the software package (McCune and Grace, 2002). Ordination was performed with the Sørenson distance measure in the Autopilot Slow and Thorough mode. The starting configuration was optimized in previous ordinations to achieve the lowest stress. A Monte Carlo test of significance as well as a Multi-Response Permutation Procedure (MRPP) was subsequently used to test for differences between *a priori* groups. All data was log transformed for statistical analyses, but was presented as untransformed data in all figures and tables.

3.5 Results

This study assessed the effect of clear cutting on soil microbial biomass, microbial community composition, bioavailability of four key plant nutrients (N, P, K, and Ca) soil moisture content, and chemical soil parameters. The same cutblock area was sampled in June 2007 (pre-harvest) and 2008-2010 (post-harvest). Additionally, comparisons were made between the LFH and Ae horizons. The effect of clear cutting is indicated here by stand age in that the pre-harvest sampling year (2007) was a 77 yr old tree stand and the post-harvest sampling years (2008–2010) were 0, 1 and 2 yr old tree stands.

3.5.1 Soil properties

Of the selected chemical soil properties, the effect of clear cutting was significantly related to pH, total N content and the C/N ratio (Table 3.1, 3.2). Compared to the 77 yr old tree stand, 1 yr post-harvest soils had lower N_{total}, a higher C/N ratio, and a slight more basic pH. Contrastingly, the effect of harvesting was not significantly related to carbon content, both C_{total} and C_{org}, or GWC, nor did they indicate a consistent trend from pre to post-harvest conditions. Soil moisture content was correlated with all of the chemical, nutrient and microbial parameters tested (Table 3.3). Soil moisture did, however, change significantly in 2010 (t=2 yrs), with a LFH moisture content of 117 % in 2010 compared to 56 % in 2009 (t=1 yr). The LFH had more moisture, carbon substrate, and N than the Ae horizon, but the pH did not differ between the two (Table 3.1, Table 3.2).

Table 3.1. Mean +/- standard deviation (n=9) of gravimetric soil water content (GWC), total carbon (C_{total}), total nitrogen (N_{total}), carbon to nitrogen (C/N) ratio, organic carbon (C_{org}), and pH of LFH and Ae soil horizons from a clear-cut site in central AB sampled in 2007 (pre-harvest), 2009 (t=1 yr post-harvest) and 2010 (t=2 yrs post-harvest).

	2007		2009		2010	
	LFH	Ae	LFH	Ae	LFH	Ae
GWC (%)	79 (8.0)	23 (0.60)	56 (17)	20 (17)	120(40)	35 (4.2)
C_{total} (%)	17 (3.0)	1.2 (0.22)	19 (7.2)	1.4 (0.34)	16 (6.2)	1.6 (0.34)
N_{total} (%)	0.85 (0.06)	0.60 (0.04)	0.51 (0.16)	0.14 (0.03)	0.50 (0.17)	0.10 (0.020)
C_{org} (%)	15 (2.8)	1.1 (0.23)	19 (6.7)	1.3 (0.35)	14 (5.8)	1.4 (0.30)
pH	4.3 (0.20)	4.6 (0.20)	5.2 (0.30)	5.4 (0.30)	4.3 (0.20)	4.9 (0.10)
C/N	16 (5.6)	3.9 (6.5)	37 (3.8)	9.6 (1.2)	30 (7.3)	17 (3.1)

Table 3.2. Kruskal-Wallis significance test of the independent grouping factors of age and soil horizon on gravimetric water content (GWC), total carbon (C_{total}), total nitrogen (N_{total}), organic carbon (C_{org}), carbon/nitrogen (C/N) ratio, pH, bioavailable N, P, K, and Ca (flux measured into the PRS^{TM} probe; proxy of soil water nutrient concentration), bacterial (B) and fungal (F) biomass, and the bacterial/fungal biomass ratio (B/F). Soil samples were from a clear-cut site in central AB sampled in 2007 (pre-harvest), 2009 (*t*=1 yr post-harvest) and 2010 (*t*=2 yrs post-harvest).

	GWC	C_{total}	N_{total}	C_{org}	C/N	pH	N	P	K	Ca	B	F	B/F
	---------- % ----------						--μg/10 cm^2/12 week burial time---				---- nmol/g ---		
Age	ns	ns	***	ns	**	***	***	***	***	*	*	ns	ns
Horizon	***	***	***	***	***	ns	***	*	**	**	***	***	***

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability level, respectively

Table 3.3. Spearman's rank correlations between gravimetric water content (GWC), total carbon (C_{total}), total nitrogen (N_{total}), organic carbon (C_{org}), carbon/nitrogen ratio (C/N), pH, bioavailable N, P, K and Ca (flux measured into the PRS^{TM} probe; proxy of soil water nutrient concentration), and total microbial biomass derived from PLFA. Soil samples were from a clear-cut site in central AB sampled in 2007 (pre-harvest), 2009 (*t*=1 yr post-harvest) and 2010 (*t*=2 yrs post-harvest).

	GWC	C_{total}	N_{total}	C_{org}	C/N	pH	N	P	K	Ca	Biomass
	----------%----------						------- μg/10 cm^2/12 week burial time -------				nmol/g
GWC	1.0	0.669***	0.530***	0.717***	0.516***	-0.341*	0.458***	0.468***	0.461***	-0.314*	0.744***
C_{total}		1.0	0.471***	0.921***	0.865***	-0.190	0.336*	0.341*	0.382*	-0.386**	0.651***
N_{total}			1.0	0.524***	0.118	-0.175	0.469***	0.330*	0.299*	-0.080	0.670***
C_{org}				1.0	0.746***	-0.210	0.402**	0.392**	0.399**	-0.401**	0.729***
C/N					1.0	-0.145	0.201	0.280*	0.263*	-0.412**	0.495***
pH						1.0	0.192	0.020	0.112	0.020	-.0125
N							1.0	0.630***	0.612***	-0.008	0.438**
P								1.0	0.568***	0.335**	0.279*
K									1.0	0.377***	0.302*
Ca										1.0	-0.397**
Biomass											1.0

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability level, respectively

3.5.2 Bioavailable nutrients

Nitrogen, P and K availability increased immediately after harvesting then decreased in subsequent years (Figure 3.1). This trend was the same in both the LFH and Ae for all three nutrients, though the initial spike in availability of N was subtle compared to P and K. The effect of clear cutting and soil horizon were both significant at $p<0.05$ (Table 3.2).

Though the effects of harvesting and soil horizon were also significant factors in Ca availability, the trend was different from that of N, P and K. Firstly, Ca availability was greater in the mineral soil than the LFH, opposite from the other nutrients, and secondly, the response to harvesting was not consistent within the two horizons as they were for the other nutrients. The LFH exhibited an increase in Ca availability after harvesting, but the mineral soil did not show any response. Calcium availability was not correlated with total soil N or N bioavailability whereas the other nutrients were (Table 3.3).

As indicated by Spearman's rank correlations in Table 3.3, all four nutrients were significantly correlated with soil water content, C_{total}, and C_{org} though Ca yielded negative correlations. Phosphorus, K and Ca availability were also significantly correlated with C/N ratio, and again, Ca was a negative correlation.

Though not assessed as part of my M.Sc., data for a suite of other nutrient fluxes were measured with PRS^{TM} probes. These data are presented in Appendix B with four graphs organized by sampling year and soil horizon.

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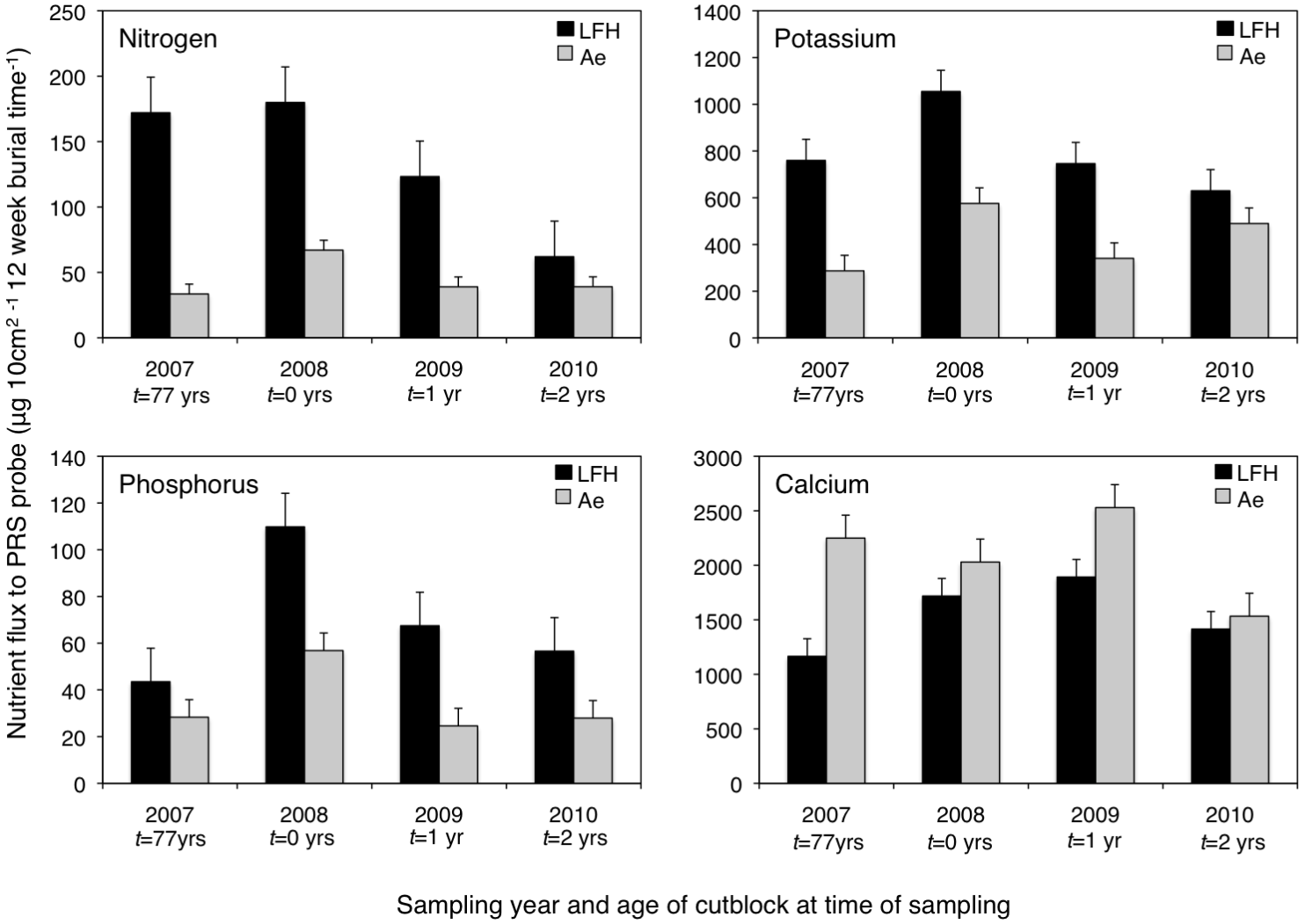

Figure 3.1 Nitrogen, potassium, phosphorus and calcium availability measured as a flux into PRS^{TM} probes from the LFH and Ae horizon from the field site in central AB. The site was sampled in June of 2007 (pre-harvest), and 2008 – 2010 (post-harvest), and was clear cut in January of 2008. The *t* indicates the age of the cutblock at the time of sampling. Error bars are standard error (n=9).

3.5.3 Microbial community biomass and composition

The total microbial biomass did not differ between pre-harvest soils in 2007 and post harvest soils in 2009 (Figure 3.2A). In 2010, however, three growing seasons after harvesting, the total biomass decreased by half. The trend in the LFH was the same as that of the Ae horizon; little change in biomass until 2010 when a significant decline ensued.

The bacterial/fungal (B/F) ratio, on the other hand, did not change significantly between 2007 and 2010 (Figure 3.2B; Table 3.2). In the LFH, there was roughly four times more bacterial biomass than fungal biomass. In the mineral soil, the bacterial biomass was even more dominant; around eight times the total fungal biomass. In either the case of total biomass or B/F ratio, there was a large difference between the community size of the LFH and Ae horizons ($p<0.001$) (Figure 3.2).

Contrastingly, the microbial community composition did change as a result of harvesting (Figure 3.3). Non-metric multidimensional scaling analysis showed that stand age separated out along the x-axis, accounting for 72% of the variability in the dataset ($p<0.0001$). Figure 3.3 B indicated that the microbial community also differed by soil horizon ($p<0.00001$); the soil horizon effect accounted for 23% of the variability of the data.

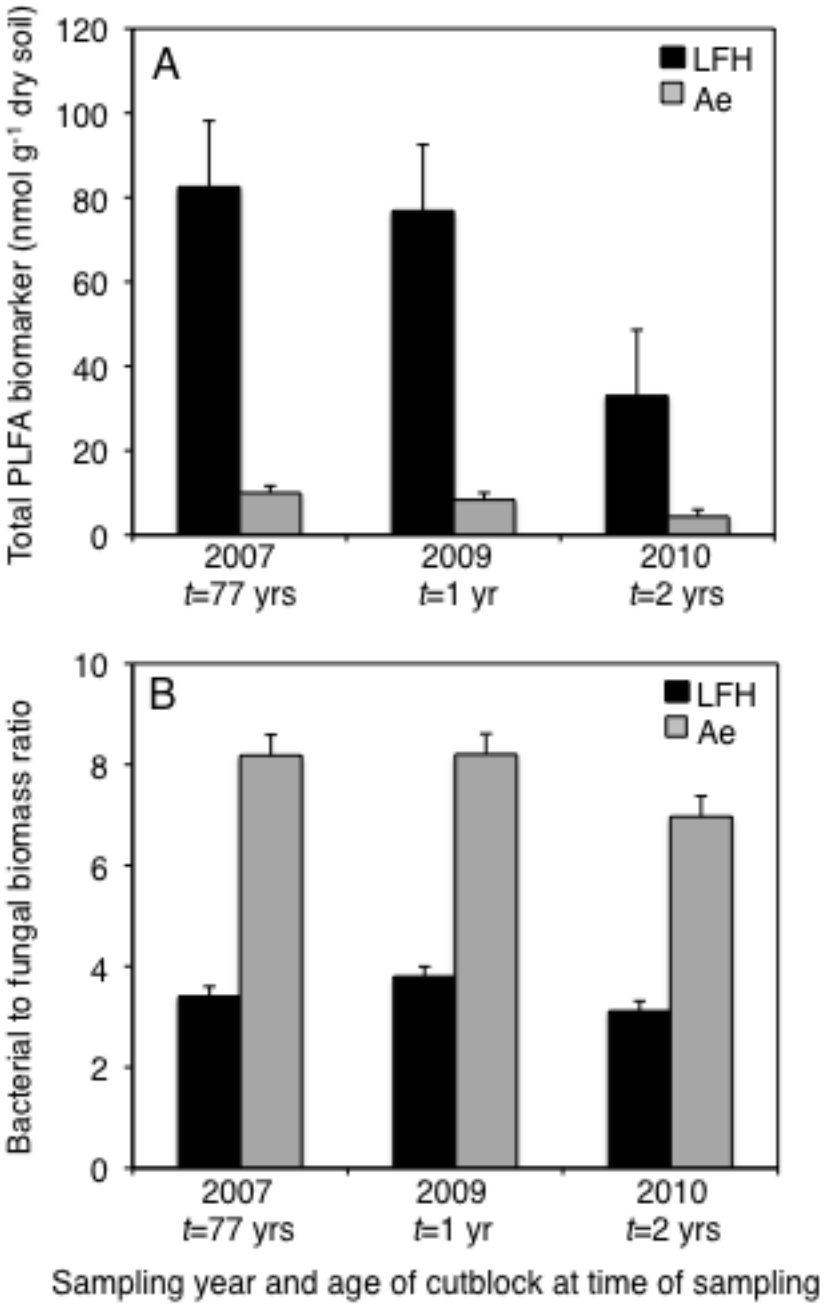

Figure 3.2. Phospholipid fatty acid biomarkers indicating total microbial biomass (A) and bacterial to fungal ratio (B) from the LFH and Ae horizon of a clear cut site in central AB, sampled as a time series in 2007 (pre-harvest; *t*= 77 yr old tree stand), and 2009 – 2010 (post-harvest; *t*= 1 and 2 yr old trees, respectively) Error bars represent standard error (n=9).

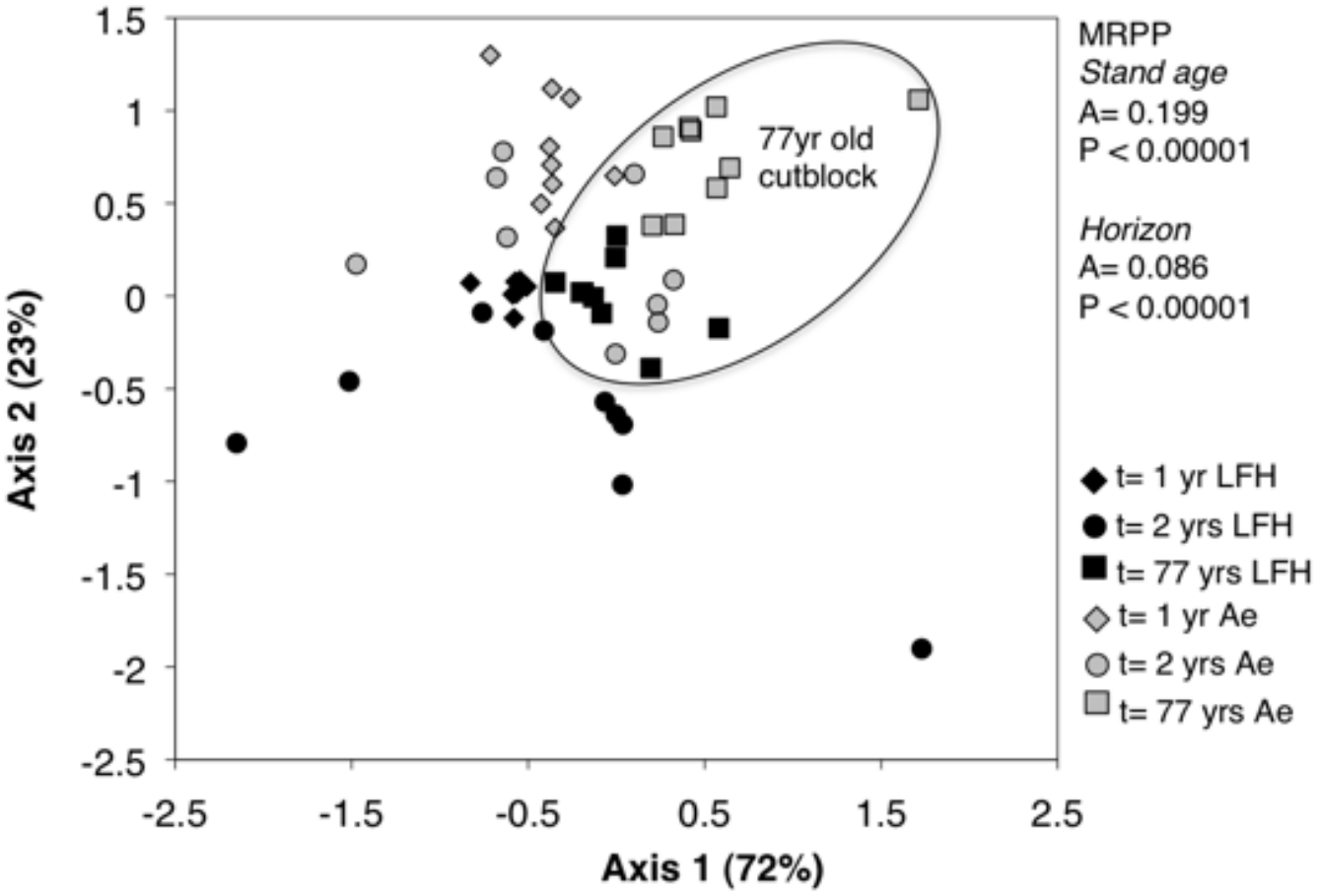

Figure 3.3. Non-metric multidimensional scaling and multiple response permutation procedure (MRPP) analysis of phospholipid fatty acid (PLFA) biomarkers from a cutblock sampled in 2007 as a 77 yr old tree stand, and after harvesting in 2009 and 2010 (t= 1 yr and 2 yrs). MRPP analysis was performed by age and soil horizon. PLFA data were analyzed in log transformed, mol% format, and the final stress was 11.2. The *A* statistic indicates within group homogeneity; an *A* value 1 means the samples within a group are identical, *A*=0 would indicate a level of homogeneity expected by chance, and an *A* of around 0.3 is typical for microbial datasets (McCune and Grace, 2002).

3.6 Discussion

3.6.1 Chemical changes incurred due to harvesting

3.6.1.1 Soil chemical parameters

Only the N_{total}, C/N ratio, and pH were significantly affected by clear cutting, whereas C_{total}, C_{org}, and soil water content were not. These results suggest that harvesting caused a relatively minor disturbance to the soil chemical conditions. Though there were measurable differences in some chemical parameters, the dataset on the whole does not suggest soil degradation resulted from these harvesting activities. This interpretation is supported by Hannam et al. (2006) who's research in a proximal location on forest-managed soils of AB also found impacts to be minimal and concluded the forestry practices to be effectively mitigating environmental degradation.

3.6.1.2 Nutrient availability

The flush of N, P and K that occurred immediately after harvesting followed by a subsequent decrease in the following years is a commonly reported trend (Grigal, 2000; Hollis et al., 1978; Keenan and Kimmins, 1993; Shrestha and Chen, 2010; Silkworth and Grigal, 1982; Simard et al., 2001). The initial increases in N, P and K may have been a result of a) the sudden cessation of tree root uptake; b) increased erosion and leaching rates commonly associated with the removal of trees and general harvesting operations (Chanasyk et al., 2003); c) increased mineralization and nitrification rates (Grigal, 2000; Keenan and Kimmins, 1993). Likely, the increase N, P and K was a result of some combination of these processes. It is generally believed that N, P and K losses are recharged over time because there are sufficient environmental inputs

to reach pre-harvest availability in the long term, though it may take 20-120 years, depending on the nutrient (Alban, 1982; Grigal, 2000; Silkworth and Grigal, 1982; Simard et al., 2001).

Calcium, on the other hand, does not have sufficient environmental inputs to be recharged post-harvest, and thus is of special concern (Alban, 1982). The response of Ca availability to harvesting was also unique in that it was different than N, P and K trends. In general, Ca availability did not change in a consistent manner in response to harvesting, and any changes that did occur were relatively small (Figure 3.1). The LFH Ca availability shifted over the four measured growing seasons differently from the Ae Ca, which was also unique from N, P and K which all trended consistently in both horizons. Calcium availability exhibited little or no change due to harvesting disturbance in other studies as well (Johnson and Todd, 1998; Silkworth and Grigal, 1982; Simard et al., 2001). Where N, P and K availability decreased over the years post-harvest, Ca stayed relatively constant or even increased. This was perhaps due to the fact that Ca inputs to the soil are predominantly mineral weathering (Alban, 1982). Though other soil constituents were experiencing a net loss from the soil, potentially due to erosion and leaching, the process of mineral erosion may have actually deposited more Ca into the soil, resulting in a slight net gain of Ca. This mineral weathering often occurs in lower mineral horizons, and some of the resultant Ca can be translocated upwards via roots to surficial soil horizons. Therefore, the increase in LFH Ca may have been a function of increased translocation from deeper soils, and the constant supply of Ca to the Ae horizon could have been a result of increased soil weathering.

3.6.2 Microbial changes incurred due to harvesting

3.6.2.1 Microbial biomass

The total size of the microbial community pre-harvest (77 yr old tree stand) was very similar to the biomass 1 yr post-harvest, suggesting that clear cutting did not cause an immediate change in microbial biomass. In addition, the soil moisture and soil carbon (both total and organic C) did not differ between pre- and one year post-harvest conditions. Since these two parameters are important determinants of microbial communities (Moore-Kucera and Dick, 2008), and neither one was impacted directly by clear cutting, it was expected that microbial biomass would not be either. The lack of change in the B/F ratio further implied that the disturbance of the microsite was relatively minor, since fungi are a sensitive indicator of disturbance (Bååth et al., 1995; Hassett and Zak, 2005). Hannam et al. (2006) and Pennanen et al. (1999) both found biomass to remain unchanged after harvesting, corroborating these results. However, by June of 2010 the microbial biomass declined significantly, and the biomass of both soil horizons reflected the same timing and magnitude of loss (Figure 3.2).

There are two plausible explanations for the decline in microbial biomass in 2010. The first line of reasoning is that the difference in soil moisture between 2009 and 2010 (20 % moisture in the Ae horizon in 2009 vs. 35 % in 2010) caused the biomass to decline. This increase in moisture corresponded with almost twice as much precipitation (ppt) being recorded at the Environment Canada Weather Station at Whitecourt AB between May 1 and June 30 2010 (153 mm in 2010 vs. 82.1 mm between the same dates in 2009) (Environment Canada, 2011). Furthermore, soil moisture was correlated with microbial biomass at a $p<0.001$, thus essentially providing a quantifiable measure of the interaction between moisture and microbial biomass. The soil moisture content in 2007 (pre-harvest) was roughly the same as that in 2009 (23% in the Ae

horizon in 2007 vs. 20% in 2009), even though three times the ppt was recorded between May 1 and June 30 2007 than over those dates of 2009 (280.1 mm in 2007 vs. 82.1 mm in 2009) (Environment Canada, 2011). The juxtaposition between the site in 2007, when there had been 280.1 mm ppt and mature trees on site, and 2009 when there was only 82.1 mm ppt and the trees had been harvested displays the critical role the mature trees played in this ecosystem. Even with three times more ppt in May – June 2007 than May – June 2009, the Ae soil moisture content was essentially the same, likely because the transpiration of the mature trees 2007 compensated for the threefold difference in ppt.

The second hypothesis for the decline in microbial biomass in 2010 is that the microbial biomass exhibited a delayed response to clear cutting. It has been suggested by Moore-Kucera and Dick (2008) that there is a 'lag time' after harvesting in which the microbial community, and especially the fungi, can thrive on the decomposing tree roots. In addition, the increase in nutrient availability also observed in this study may have provided the microbial community with additional resources to thrive on, thereby sustaining the same population size as pre-harvest conditions did. By 2010, nutrient availability had begun to decline (Figure 3.1) and the decomposing roots may not have not been able to provide enough sustenance for the same population size as in the previous two growing seasons, therefore, there was a decline in microbial biomass by June of 2010, two years after the site was harvested.

3.6.2.2 Microbial community composition

There was a clear difference in the community composition before vs. after harvesting based on PLFA analysis, and the change that occurred due to harvesting represented 72% of the variation between 2007 and 2010 (Figure 3.3). The chemical properties of the site after

harvesting were different than pre-harvest; there was a greater availability of N, P, K and Ca, the pH was slightly less acidic, and C/N ratio increased (Table 3.1, 3.2, Figure 3.1). Some combination of these changes caused the community to shift towards microorganisms better adapted to the new microsite conditions.

External to this study, but of relevance in this ecosystem, the shift in plant cover that occurred immediately after cutting, and continually over the subsequent years, likely influenced the microbial community composition as well (Boyle-Yarwood et al., 2008). Harvesting results in a change in plant species composition as well as altering the age-class distribution of the ecosystem (Grigal, 2000). These changes inflict a whole host of alterations to soil substrate and microsite characteristics ranging from root interactions with fungi (Jones et al., 2003), quality and quantity of root exudates (Moore-Kucera and Dick, 2008), competition for nutrients (Keenan and Kimmins, 1993), and the quality and quantity of organic matter deposited from the above ground biomass (Attiwill and Adams, 1993). As a result, this new set of environmental conditions selected for different soil organisms than those that were successful in the pre-harvest microsite conditions.

One of the challenges of studying community composition in the context of environmental disturbances is that it is difficult to draw connections between microbial 'form and function'; i.e. between which microbes are present and how their activity directly relates to soil productivity. Ultimately, the change observed in community composition due to harvesting indicated that the microsite was changing, and the soil ecosystem was responding accordingly. Maintaining a high microbial diversity is important, especially in disturbed areas, to act as a 'buffer' to perturbations. As seen here, when a disturbance takes place, the community shifts to those better adapted, and the functionality of the ecosystem continues, albeit being carried out by

a different set of organisms. In this way, a shift in community composition is a healthy ecosystem response; the soil community is resilient enough to adapt to changing conditions. The mere fact that a change in the community is measurable does not necessarily mean that it was detrimental.

3.6.3 Comparison of LFH versus Ae

The Ae horizon of these forest soils was significantly different from the LFH in each chemical and microbial parameters measured, except for pH. Total biomass was markedly lower and the fungal community more depleted in the Ae relative to the LFH (Figure 3.2). Community composition differed and bioavailability of N, P and K were drastically smaller than those of the LFH horizon. Mineral Ae soil was significantly drier, lower in carbon (both total and organic) and total N, slightly more basic (0.2-0.6 pH units), and had lower C/N ratios (Table 3.1). Since all of these life-supporting soil parameters as well as fresh organic material (labile substrate) were in shorter supply in Ae soils (compared to the LFH), it could not sustain as large a population as the LFH, resulting in a smaller microbial biomass, and lower nutrient availability. Fungal biomass was especially depleted in the Ae soil because fungi are primarily decomposers, and with less decomposable material, fewer organisms can thrive. Furthermore, competition for resources would be greater in a more limited environment which would keep the total population size down (Mariani et al., 2006). The composition of the microbial community was distinct from that in the LFH because of the differences in the soil parameters; a more nutrient, water and substrate limited environment with greater competition would select for different organisms than the abundant, moist, conditions of the LFH soil.

Importantly, the parameters of the LFH and Ae soil illustrated the same trends and responses to harvesting, corroborating the results of Simard et al. (2001). Most forest soil studies sample only one soil horizon, or homogenize a certain amount of soil regardless of which horizon it originated from. One of the objectives was to assess the differences between the two horizons, so that I could conclude whether it is actually accurate to measure only one horizon, or a homogenization of two. Upon comparing microbial biomass, B/F ratios, community composition, soil chemical and bioavailable nutrients response to clear-cutting in the LFH and mineral soil separately I can conclude that, indeed, sampling one horizon or homogenizing would yield trends accurate of either horizon independently (except for Ca availability). Thus, this comparison of the two surficial forest soil horizons indicates that for objectives seeking relative comparisons, homogenization is sufficient, but for those seeking absolute values, separate horizons should be analyzed separately.

3.7 Conclusions

This study indicated that harvesting of these Boreal forest soils had a relatively minor impact on soil chemical parameters. Microbial biomass was not diminished in the first growing season after clear cutting, but community composition was. It is unclear whether the community exhibited a delayed response to harvesting, or was impacted by a doubling of moisture content in 2010. In either case, this study captured the important role mature trees have on controlling soil moisture, given the variability of precipitation over the study period yet consistency of soil moisture content from 2007 to 2009. Nutrient loss depicted a typical trend of an initial flush or increase after harvesting, followed by a subsequent loss from the soil in the three years post-harvest. The microbial community composition shifted due to harvesting, suggesting a resilient community that was capable of adapting to a different microsite conditions.

Studies of microbial communities in disturbed environments highlight the need for future research to draw connections between the 'form' of the microbes and their 'function' in the ecosystem. A comparison of the LFH and mineral Ae soil at our site suggests that the two horizons responded similarly to harvesting disturbance for all soil and microbial parameters measured, except for Ca availability. These results, and those of others (Hannam et al., 2006; Pennanen et al., 1999; Ponder and Tadros, 2002) show that best practices in the forestry industry are moving towards mitigating environmental impacts on the soil chemical and microbial ecosystem. However, loss of microbial biomass and nutrients from a clear cut site in this study represent issues that may still need to be addressed to maintain the sustainability of the forestry industry in Canadian Boreal forest soils.

4.0 A CHRONOSEQUENTIAL APPROACH TO INVESTIGATING MICROBIAL COMMUNITY SHIFTS IN THE YEARS FOLLOWING CLEAR CUTTING

4.1 Preface

In Chapter 3, it was determined that microbial biomass was impacted two years after harvesting, and different hypothesis to explain the biomass loss explored. This chapter builds upon that discussion by scaling-up to a longer time frame using a chronosequence approach of six cutblocks; all similar except for the year they were harvested. Molecular techniques were utilized in this study to investigate microbial community size and composition with genetic tools in addition to PLFA. However, very few studies have assessed the impact of harvesting using molecular tools, thus a second aspect of this chapter was to compare the PLFA fingerprinting results with the molecular results. The objective of this study was to determine whether microbial community biomass and composition changed along a chronosequence of ~20 yrs post-harvest, and whether community level (PLFA) and phylogenetic (16S rDNA) fingerprinting methods yielded the same conclusions. My general hypothesis was that microbial biomass would be smaller in younger cutblocks, and the fungal community most impacted by the disturbance, relative to bacteria. I also postulated that the community composition would differ along the chronosequence.

4.2 Abstract

The impacts of forest harvesting are often assessed in short term studies that ignore the longer term changes associated with the disturbance. A chronosequence approach was taken in this study to investigate the changes occurring in microbial community size and composition over ~20 yrs post-harvest. Six cutblocks similar to each other except for their age since harvesting were sampled in the summer of 2009 and 2010 in both the LFH and mineral Ae horizons of Orthic Gray Luvisols. Two microbial fingerprinting methods were utilized in order to capture community level changes (PLFA) and phylogenetic changes of bacteria (16S rDNA analysis). Soil properties were also measured in order to delineate microsite changes that may have played a role in microbial community shifts.

Results indicated that total microbial biomass was unaffected by harvesting disturbance, though fungal biomass was significantly larger in the oldest cutblock of the chronosequence. The doubling of soil moisture content in the summer of 2010 indicated that moisture was a stronger determinant of microbial biomass than harvesting operations. Microbial community composition did, however, differ between younger and older cutblocks as indicated by both lipid biomarker and DNA fingerprinting techniques. Forest soil microbial communities subject to clear cutting are generally resilient to the disturbance as shown by the unchanged total microbial biomass. Building upon that, the shift in community composition, which occurred in concert with the maintenance of biomass, indicated that the microbial community adapted sufficiently to the new post-harvest microsite conditions.

4.3 Introduction

The forestry industry, like the agriculture industry, relies heavily on productive soils in order to yield fast growing, healthy plants. Foresters, however, do not replace soil organic matter and nutrients as farmers do. This can result in soil degradation of long-term forestry areas (Grigal, 2000), which can ultimately decreases the rate of tree growth and quality of trees produced. This unsustainable manner of managing forest areas has been identified, and research projects like The Forest Watershed and Riparian Disturbance (FORWARD) Project have been created to address soil degradation, as well as many other ecosystem disturbances related to forestry and wildfire. In order to mitigate losses in soil productivity, we must further our understanding of how the soil ecosystem responds to harvesting, and more importantly, how it recovers in the subsequent years after the disturbance.

During the recovery years after clear cutting, various ecosystem changes have been documented. Most obviously, the vegetation cover changes dramatically; the age-class distribution as well as species composition are altered immediately at the time of harvesting and continues to change in the subsequent years, as the regular course of plant succession ensues (Grigal, 2000). Changes in the diversity of plant species and the quality/quantity of organic matter inputs (surficial, roots, and exudates) and competition dynamics all contribute to a succession of soil microsite. Physical changes to the soil microsite like increased moisture, temperature, and compaction (Hassett and Zak, 2005) may also evolve in the years following clear cutting.

Microorganisms are of central importance in the forest soil ecosystem because they facilitate organic matter decomposition and nutrient cycling in the soil (Pennanen et al., 1999).

Because they drive biogeochemical cycles, and also directly aid in plant growth and nutrition, e.g., mycorrhizae, microbial response to harvesting is a very important component of the ecosystem to understand. Many studies show that harvesting impacts either the microbial biomass (Hassett and Zak, 2005), community composition (Bäckman et al., 2004; Moore-Kucera and Dick, 2008; Niemela and Sundman, 1977; Pennanen et al., 1999; Smith et al., 2008), or both (Bååth et al., 1995), but these studies often focus on a short period of time after disturbance. A few longer-term assessments indicate that the microbial community composition returns to a similar structure as the pre-harvest community anywhere between 7-25 years after clear cutting (Moore-Kucera and Dick, 2008; Niemela and Sundman, 1977), though some find the microbial community to be resilient to harvesting disturbance all together (Hannam et al., 2006; Ponder and Tadros, 2002; Shestak and Busse, 2005).

Advances in molecular techniques have added to the 'toolbox' of means with which researchers can assess microbial populations from field settings. Though phospholipid fatty acid analysis (PLFA) is still a very useful method of assessing the viable microbial biomass and structure at a community level (Bligh and Dyer, 1959; Frostegård and Bååth, 1996; Zelles, 1999), molecular techniques like polymerase chain reaction coupled with denaturing gradient gel electrophoresis (PCR-DGGE) provide a more specific look at the community at a species level (Muyzer et al., 1993).

Scientists have made progress in this area of research, but there still remains a knowledge gap as to how the microbial community in a recently harvested sites (1-5 yrs post-harvest) compares to the community of a ~20 yr post-harvest site. Furthermore, very few studies have utilized molecular techniques to assess microbial impacts of harvesting (Bäckman et al., 2004; Smith et al., 2008; Yeager et al., 2005); most often PLFA has been used. With that in

mind, the aim of this study was to investigate changes in microbial community size and composition occurring over ~20 years of succession post-harvest. Two methods of fingerprinting, PLFA and PCR-DGGE were used to assess community composition, and community size was determined by PLFA as an indicator of biomass. Six cutblocks of similar pre-harvest conditions but harvested at different times (1-19 years post-harvest) were sampled from both the LFH and topmost mineral horizon (Ae) in this chronosequence study in the Boreal Plain of central Alberta.

4.4 Materials and Methods

4.4.1 Site description

The study site was located in central Alberta (54°03' N, 115°84' W; 782 masl) on the Boreal Plain, 220 km northwest of Edmonton, Alberta. The region is underlain by Cretaceous and Tertiary sandstones, shales, clays and gravels ranging from <15 to >150 m thick (Pawlowicz and Fenton, 1995). The predominant soil type is Orthic Gray Luvisol, which typically exhibits an organic LFH layer, an Ae horizon leached of clay, and a Bt horizon enriched in clay from the above Ae horizon (Agriculture Canada Expert Committee on Soil Survey 1987). The predominant trees in the area are lodgepole pine (*Pinus contorta*), though white spruce (*Picea glauca*), balsam poplar (*Populus balsamifera*) trembling aspen (*Populus tremuloides*), jack pine (*Pinus banksiana*), and black spruce (*Picea mariana*) are also found (Smith et al., 2003).

Six cutblocks, each harvested during January of a different year (Table 4.1) were sampled for this chronosequence study. Post-harvest site preparation consisted of the rake and burn method in which large branches and debris are collected and burned (during a wet period to avoid wildfire). Small branches and debris were left on site to decompose. During the first or second growing season after clear cutting, each cutblock (except for the site clear cut in 1991) was treated with Vision (Monsanto Inc; active ingredient glyphosate) at a rate of six liters per hectare. Cutblocks were re-planted with lodgepole pine seedling plugs in the first growing season after harvesting.

Table 4.1. General information of the cutblocks sampled for this study. All six sites were clear cut in January of their respective harvest years, were predominantly Orthic Gray Luvisols, and were prepared by the rake and burn method. All six were re-planted with lodgepole pine seedling plugs in the first growing season following clear cutting.

Cutblock ID	Year of clear cutting	'Age' of cutblock (years)		Lodgepole cover † (%)	Vision application	Slope of site (%)	LFH thickness (cm) ‡
		2009	2010				
OCE 222	2008	1	2	90	2010	1.5	5.3 (2.4)
OCE 153	2007	2	3	80	2007	15	8.0 (7.5)
OCE 136	2006	3	4	60	2008	16	4.8 (1.8)
OCE 112	2005	4	5	90	2007	1	7.0 (4.0)
OCE 116	2004	5	6	90	2006	2	5.6 (2.2)
W5L4-73	1991	18	19	NA	NA	5	7.8 (9.6)

† Before being harvested
‡ Standard deviations in brackets

The region experiences mean summer and winter temperatures of around 10 °C and -15 °C, respectively, and an average of 500 – 600 mm of precipitation (ppt) annually (Environment Canada, 2000; Strong and Leggat, 1992). Though this is the average, it is of relevance to also report the total precipitation recorded in the area over the winter and spring season during which sampling occurred in June. The total ppt from October 1 – June 30 2009 was 260 mm, and during the same period of 2010 was 253 mm. However, the total rainfall recorded from May 1 – June 30 2009 was 82.1 mm, while that number for the same period in 2010 was 153.3 mm.

4.4.2 Soil sampling methodology

The cutblocks were divided into a systematic sampling plan and sampled in June 2009 and 2010. Each cutblock was separated into three transects, each transect with three sample plots, and each plot a composite of four randomly chosen subsamples (Figure A.1). Soil was collected using a JMC Backsaver probe (Clements Assoc. Inc, Newton, IA) with a 3.2 cm diameter tip. Samples were gathered from the forest floor and mineral horizon, hereafter referred to as the LFH and Ae, respectively at each sample location for a total of 108 samples per year (i.e., 3 plots x 3 transects x 2 horizons x 6 cutblocks = 108 samples per year) (Appendix A, Figures A.1-A.3). Sampling took take place along the slope gradient (Table 4.1) to account for any differences associated with slope position. Soils were stored on ice until returning to the lab, at which point they were sieved with a <2mm mesh and stored at 4 °C. One set of sub-samples was air-dried and pulverization for general soil characterization, a second set was stored at -80 °C for subsequent DNA extraction and amplification, and a third set freeze dried and stored at -20 °C for phospholipid fatty acid analysis.

4.4.3 Characterization of soil properties

All soil analyses were carried out based on the Soil Sampling and Methods of Analysis Book (Canadian Society of Soil Science, 2008). Gravimetric water content (GWC) was determined by calculating the mass lost after drying a known quantity of soil at 105 °C for 48 h, and expressed as mass of water per unit mass of dry soil (%). Soil pH was measured in a 2:1 soil: double deionized water slurry. Total C (C_{total}) and N (N_{total}) were quantified by the combustion method using a LECO CNS-2000 and expressed as a percentage (%) of the total soil mass (Helgason et al., 2009). The carbon to nitrogen (C/N) ratio was calculated by dividing the C_{total} by the N_{total} and thus reported here unit-less.

4.4.4 Phospholipid fatty acid (PLFA) analysis

Lipid extraction

Phospholipid fatty acid analysis (PLFA) was performed based on the method of Helgason et al. (2009), which was developed from the original method of Bligh and Dyer (1959). Sieved, lyophilized soil was weighed to 4.0 g and extracted in a methanol/chloroform mixture, then dried down under N_2 flow. Neutral, glyco- and phospho- lipids were separated out using solid phase extraction columns (0.50 g Si; Varian Inc. Mississauga ON), eluted with chloroform ($CHCl_3$), acetone ($(CH_3)_2CO$) and methanol (MeOH) respectively, and the phospholipid fraction dried under N_2 flow. The phospholipid fraction was then methylated in a solution of 1:1 methanol/toluene and methanolic potassium hydroxide (KOH) at 35°C.

Analysis of PLFAs

The resulting fatty acid methyl esters (FAMEs) were analyzed using a Hewlett-Packard 5890 Series II gas chromatograph with a 25-m Ultra 2 column (J&W Scientific, Mississauga, ON) in 2009. Peaks were identified using fatty acid standards and MIDI identification software (MIDI, Inc., Newark, DE). In 2010, FAMEs were analyzed using a Varian 3900 gas chromatograph with a 50-m Capillary Select FAME column (Varian, Mississauga, ON). Peaks were identified based on comparison to known standards (Supelco Bacterial Acid Methyl Esters and MJS Biolynx). All samples were quantified based on comparison with an internal standard, methyl nonadecanoate (c19:0). Comparability of 2009 data (run on the Hewlett-Packard GC) and 2010 data (run on the Varian GC), was confirmed by running a host (n=20) of identical samples on both GC's, carrying out all data analysis, and comparing final biomass calculations to each other. Total biomass of each community group from each GC was within 98% of the other (data not shown), thus it was deemed acceptable to compare 2009 data with 2010.

Biomarkers used

Bacterial biomass was determined as the sum of 10 fatty acid biomarkers, 3OH-12:0, a-12-meth-15:0, i-13-meth-15:0, 15:0, 2OH-14:0, i-14-meth-16:0, i-15-meth-17:0, 10-methyl-17:0ω8c, 17:0, and 2OH-16:0 (Hamel et al., 2006). Saprophytic fungal biomass was represented by the biomarker 18:2ω6c (Frostegård and Bååth, 1996), and arbuscular mycorrhizal fungi by the biomarker 16:1ω5 (Spring et al., 2000).

All biomass data reported here represent dry soil weight, and is reported in units of nmol/g soil derived from individual molecular weights of each fatty acid.

4.4.5 Analysis of the universal bacteria gene, 16S rDNA

DNA extraction

Total DNA was extracted from 0.5g of both LFH and Ae soil using the FastDNA Spin Kit for Soil (MP Biomedicals, Montreal QC). Cell lysis was performed by vigorous shaking using a bead beater according to manufacturer instructions. DNA extract quality was evaluated by electrophoresis on a 1% agarose gel stained with CybrSafe (Invitrogen, Burlington ON) according to manufacturers instructions and visualized on a Bio-Rad Gel Doc XR System with Image Lab Software (Bio-Rad, Mississauga, ON).

16S amplification

From the DNA extract, the universal bacteria gene 16S rDNA was amplified using primer set U341f-gc (5'-GCG GGC GGG GCG GGG GCA CGG GGG GCG CGG CGG GCG GGG CGG GGG _CCT ACG GGAGGC AGC AG-3') and U758r (3'-CTACCAGGG TATCTAATCC-5') (~417 bp fragment) (Helgason et al., 2010; Phillips et al., 2006). The optimized PCR reaction recipe consisted of 1 μL 1:10 diluted DNA extract; 0.5 μM of primers U341-gc and U758; 200 μM of each dNTP; 1.70 mM $MgCl_2$; 6.25 μg BSA (Amersham Biosciences); and 2.5 units of Platinum Taq Polymerase in 5 μL 10 × reaction buffer (Invitrogen); 31.93 μL of ddH_2O to make a total reaction volume of 50 μL.

Amplification of the targeted gene was accomplished using touchdown PCR. Ten cycles of 1 min denaturing at 94 °C, 1 min annealing at 65 to 55 °C and 1 min extension at 72 °C were carried out, followed by 18 repeated cycles using an annealing temperature of 55 °C (Helgason et al., 2010). Four of the six sites were selected for amplification and subsequent molecular work in order to achieve a manageable number of total samples, based on significant differences identified by the PLFA results.

PCR product was confirmed on a 1 % agarose gel stained with CybrSafe (Invitrogen, Burlington, ON) according to manufacturers instructions and visualized on a Bio-Rad Gel Doc XR System with Image Lab Software (Bio-Rad, Mississauga, ON). Combined product was then concentrated overnight at -20 °C with 0.1 × total volume of 3 M sodium acetate and 2.5 × total volume of 70 % ethanol to remove superfluous PCR ingredients. Concentrated DNA was re-suspended in TE buffer (Tris and EDTA mixture), and final DNA concentration determined using a NanoDrop2000 Spectrophotometer (Thermo Scientific, Ottawa, ON) and corresponding software.

Denaturing Gel Gradient Electrophoresis

Denaturing Gel Gradient Electrophoresis was then performed on 16S rDNA PCR product using a Bio-Rad DCode system (Bio-Rad, Mississauga, ON). Approximately 600 ng of DNA from each sample was loaded onto an 8 % polyacrylamide gel with a 40 – 60 % denaturing gradient of formamide and urea. Electrophoresis was carried out for 16 hrs at 80 V and 60 °C. The resulting gels were stained with CybrSafe (Invitrogen, Burlington, ON) in 1 × TAE buffer (Tris, acetic acid and EDTA mixture) for 0.5 hrs, de-stained for 0.5 hrs and photographed using a Bio-Rad Gel Doc XR System with Image Lab Software (Bio-Rad, Mississauga, ON). Using a sterile scalpel, random dominant bands were excised and eluted in H_2O for 45 min at 37 °C. Eluted DNA was re-amplified using the same primers and temperature program as above. Confirmation of re-amplified fragments was carried out on 2 % agarose gel, followed by excision and purification with the GeneClean II Kit (Qbiogene, Carlsbad, CA) by manufacturers instructions. Finally, DNA sequences were obtained from the Plant Biotechnology Institute (NRC, Saskatoon, SK) and submitted to the GenBank database in order to confirm bacterial origins.

4.4.6 Statistical analysis

Significance testing

Data exploration, correlations and significance testing to compare groups was carried out with SPSS Version 17.0 for Windows (SPSS Inc., 2008). Log transformed chemical and microbial soil parameters were tested for normality (Shapiro-Wilks) and homogeneity of variance (Levene's Test), and were determined to be non-normal and heteroscedastic, thus statistical tests that do not violate parametric assumptions were used to analyze the data (Field, 2005). The Kruskal-Wallis test, which compares independent groups, was used to analyze the effect of clear-cutting, soil horizon and sampling year on soil and microbial parameters, and correlation coefficients were derived using Spearman's rank correlations for non-parametric data.

Analysis of DGGE gels

Band detection, gel analysis and cluster analysis for the PCR-DGGE component was carried out in Bionumerics v.5.1 software (Applied Maths, Austin, TX). DGGE band identification was set for a least square filtering cut-off below 1.38% and background subtraction (disk size) of 9.63%. Band selection utilized a minimum profiling of 5%, a position tolerance of 0.87% and with optimization of 0.55%. Based on densiometric curves and the Ward linkage method, cluster analysis was performed using the Pearson correlation coefficient. A binary presence-absence matrix was then created by bandmatching created from the densiometric curves (Helgason et al., 2010; Peixoto et al., 2006)

Ordination

Non-metric multidimensional scaling (NMS) analysis using PCOrd v.5.0 (MjM Software

Gleneden Beach, OR) was used to analyze the community composition of the PLFA biomarkers and 16S rDNA DGGE band presence-absence matrix (Helgason et al., 2010; Smith et al., 2008; Swallow et al., 2009). This method of ordination is appropriate for ecological datasets such as this one because it is nonparametric, thus can handle non-normally distributed ecological data (McCune and Grace, 2002). Phospholipid data was expressed as mol% and was transformed using the log (mol% + 1) transformation as recommended in the software package (McCune and Grace, 2002). Ordination was performed with the Sørenson distance measure in the Autopilot Slow and Thorough mode. The starting configuration was optimized in previous ordinations to achieve the lowest stress. A Monte Carlo test of significance as well as a Multi-Response Permutation Procedure (MRPP) was subsequently used to test for differences between independent groups. All data was log transformed for statistical analysis, but is presented as untransformed data in all figures and tables.

4.5 Results

The purpose of this study was to investigate changes in soil microbial communities in the years following clear-cutting using a chronosequence approach. Six cutblocks of the same soil type and pre-harvest vegetation but harvested in different years were sampled in June of 2009 and 2010 from the LFH and Ae horizons. Cutblocks are designated by 'age' where *t* indicates years since harvesting. As is the nature of a chronosequence study, the fundamental assumption is that trends/relationships observed are a function of time since harvesting, and not of differences between sites. Sites were carefully selected based on similarity of soil type, pre-harvest vegetation cover, etc. in order to ensure the validity of interpretations made of the chronosequence, as has been done elsewhere (Griffin et al., 2011; Moore-Kucera and Dick, 2008; Simard et al., 2001).

4.5.1 Soil properties

Over ~20 years after clear-cutting at these sites, the soil moisture content, pH and C/N ratio changed significantly in relation to time since harvesting (Table 4.2). The C/N ratio was significantly higher for the younger cutblocks compared to the ~20 yr old site in the LFH ($p<0.01$), and this was consistent for both sampling years, but the C/N of the Ae horizon did not change over the chronosequence. The pH of the ~20 yr old site was slightly less acidic than the soil of younger sites, but this trend existed only in the 2009 samples. In both sampling years, the Ae horizon was significantly less acidic than the LFH at a $p<0.01$ (Table 4.2, 4.3). Total carbon (C_{total}) and nitrogen (N_{total}) indicated no trend in relation to stand age (Table 4.2). More recently harvested soils were generally wetter than older cutblocks, and the LFH wetter than Ae soil (Table 4.2, Figure 4.1). There was a marked difference in soil moisture between the 2009 and

2010 growing seasons. In 2010 the soil held roughly twice the moisture as in 2009 (Figure 4.1). This moisture content was reflective of ppt data gathered from the spring of each year, which was 54% greater in 2010 than 2009. The time period of ppt measured was chosen to be May 1 to June 30 of each sampling year to reflect the spring rainfall, which was the time period most likely to have a direct influence on the microbial communities sampled in June. In May and June of 2009, 82.1 mm of ppt were recorded, whereas in 2010, 153.0 mm were recorded over the same time period (Environment Canada, 2011). The disparity between moisture content of the two sampling summers was statistically represented by the independent factor of the 'effect of sampling year'. In this respect, sampling year, i.e. moisture content, caused significant differences in soil pH and, obviously soil moisture at a $p<0.001$ (Table 4.2). In regards to generalized comparisons of the LFH versus Ae characteristics, all parameters measured except pH were significantly lower in the Ae soil than the LFH (Table 4.2, 4.3).

Table 4.2. Kruskal-Wallis significance test of the independent grouping factors of age, soil horizon and sampling year on gravimetric water content (GWC), total carbon (C_{total}), total nitrogen (N_{total}), carbon/nitrogen (C/N) ratio, pH, bacterial (B), fungal (F) and arbuscular mycorrhizal fungal (AMF) biomass. Soil samples were from the LFH and Ae horizon of a clear-cut area in central AB sampled in 2009 and 2010 from sites of various ages post-harvest ranging from *t*= 1 to 19 yrs post-harvest.

	GWC	C_{total}	N_{total}	C/N	pH	B	F	AMF
	---------- % ----------					---------- nmol/g ----------		
Age	*	ns	ns	**	***	ns	**	ns
Horizon	**	***	***	***	**	***	***	***
Sampling year	***	ns	ns	ns	***	***	***	***

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability level

Table 4.3. Mean +/- standard deviation total carbon (C_{total}), total nitrogen (N_{total}), carbon/nitrogen ratio (C/N), and pH of LFH and Ae soils from cutblocks from central Alberta. Cutblocks were harvested in the year (HY) 2008 through 2004 and in 1991, and sampled in June of 2009 and 2010.

	2009 Sampling Year				2010 Sampling Year			
	C_{total}	N_{total}	C/N	pH	C_{total}	N_{total}	C/N	pH
	--------% ---------				--------% ---------			
				LFH				
t=1 yr HY 2008	19 (6.7)	0.51 (0.16)	37 (3.8)	5.2 (0.30)	16 (6.2)	0.50 (0.17)	30 (7.3)	4.3 (0.20)
t=2 yrs HY 2007	17 (6.7)	0.65 (0.33)	28 (6.4)	6.0 (0.40)	18 (5.9)	0.78 (0.22)	24 (5.1)	5.1 (0.80)
t=3 yrs HY 2006	16 (6.5)	0.47 (0.16)	33 (3.8)	6.0 (0.20)	20 (6.3)	0.77 (0.29)	25 (4.7)	4.6 (0.70)
t=4 yrs HY 2005	18 (6.7)	0.51 (0.18)	37 (6.4)	5.6 (0.20)	13 (4.8)	0.54 (0.15)	23 (5.5)	4.9 (0.20)
t=5 yrs HY 2004	18 (6.6)	0.53 (0.18)	34 (7.2)	5.2 (0.40)	16 (8.9)	0.37 (0.16)	43 (16)	4.4 (0.30)
t=18 yrs HY 1991	11 (3.0)	0.51 (0.16)	21 (3.0)	6.4 (0.50)	9.4 (3.2)	0.61 (0.12)	15 (4.0)	4.9 (0.20)
				Ae				
t=2 yrs HY 2008	1.3 (0.35)	0.14 (0.030)	9.6 (1.2)	5.4 (0.30)	1.6 (0.34)	0.10 (0.020)	17 (3.1)	4.9 (0.10)
t=3 yrs HY 2007	1.5 (0.52)	0.22 (0.20)	8.1 (2.1)	6.1 (0.30)	0.19 (0.090)	0.050 (0.050)	4.9 (1.8)	5.3 (0.60)
t=4 yrs HY 2006	1.6 (0.48)	0.14 (0.030)	11 (1.1)	6.6 (0.40)	1.8 (0.50)	0.20 (0.030)	9.0 (2.2)	4.9 (0.60)
t=5 yrs HY 2005	1.3 (0.25)	0.12 (0.020)	11 (1.5)	6.3 (0.20)	1.7 (0.52)	0.18 (0.16)	15 (8.4)	5.1 (0.30)
t=6 yrs HY 2004	1.3 (0.54)	0.13 (0.040)	11 (3.5)	5.8 (0.20)	1.4 (0.27)	0.21 (0.040)	6.9 (1.3)	4.8 (0.40)
t=19 yrs HY 1991	1.5 (0.51)	0.15 (0.040)	9.5 (1.0)	6.5 (0.10)	2.1 (0.51)	0.24 (0.060)	8.5 (1.5)	5.2 (0.60)

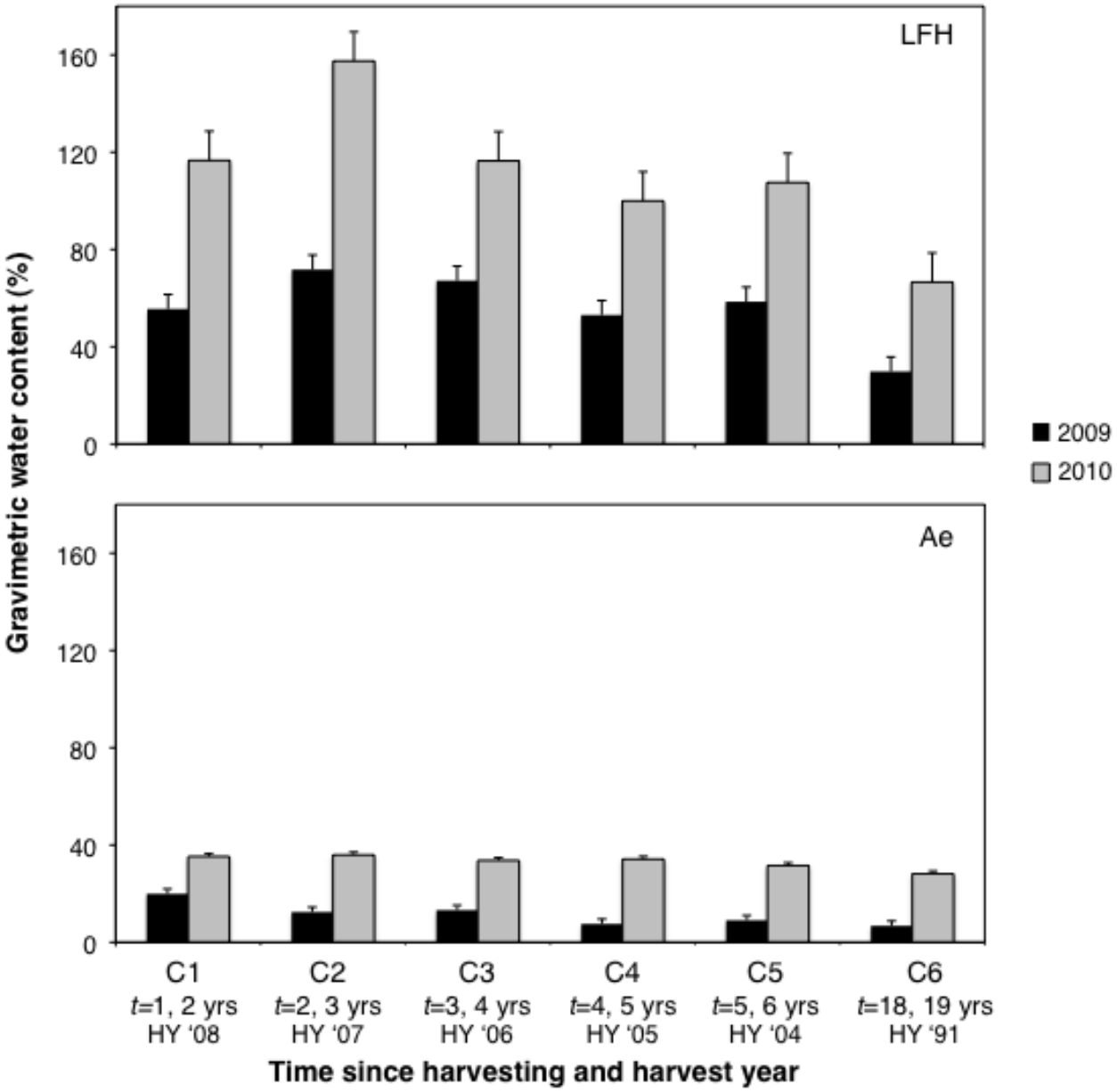

Figure 4.1. Mean +/- standard error; (n= 9) gravimetric soil water content of the LFH (top) and Ae (bottom) horizon of soil samples from six cutblocks (C), each harvested in a different year (HY), ranging from 2008-1991, sampled in June of 2009 and 2010. Total precipitation from May 1 – June 30 2009 was 82 mm, and 153 mm from the same period of 2010; 54% more rainfall was added to the soil in the spring of 2010.

4.5.2 PLFA derived microbial biomass

The saprophytic fungi were the only group whose biomass changed in relation to stand age ($p<0.01$), and this relationship was driven by the large fungal community size in the ~20 yr old stand in the 2009 samples (Table 4.2, Figure 4.2). In 2009 samples, the saprophytic fungal biomass was largest in the oldest cutblock, of an intermediate size in the most recently harvested cutblock, and relatively depleted in the 2 – 5 yrs post-harvest cutblocks. Bacterial and arbuscular mycorrhizal fungal (AMF) biomass did not fluctuate throughout the chronosequence in relation to time since harvesting. There was much more bacterial biomass in these soils than saprophytic fungal biomass, and roughly three times more saprophytic fungal biomass than AMF biomass. All three groups including saprophytic fungi, bacteria, and AMF, had significantly larger communities in the LFH than Ae soil.

In 2010, there was a stark decline in the biomass of all community groups. The intra-group trends differed in 2010 soils as well; biomass was greatest at the youngest site for all three communities, and this was not the case in the 2009 samples.

4.5.3 PLFA biomarker derived community composition

Microbial community composition differed significantly by stand age and soil horizon when analyzed by NMS and MRPP (Figure 4.3). The microbial community in the ~20 yr old cutblock was distinct from the younger sites' community composition in both 2009 and 2010 soils, though the distinction was more clear in 2009 soils. The within group variance, indicated by the *A* statistic, revealed that there was more homogeneity within each group (age and horizon) than expected by chance (McCune and Grace, 2002). In 2009 samples, the C/N ratio was

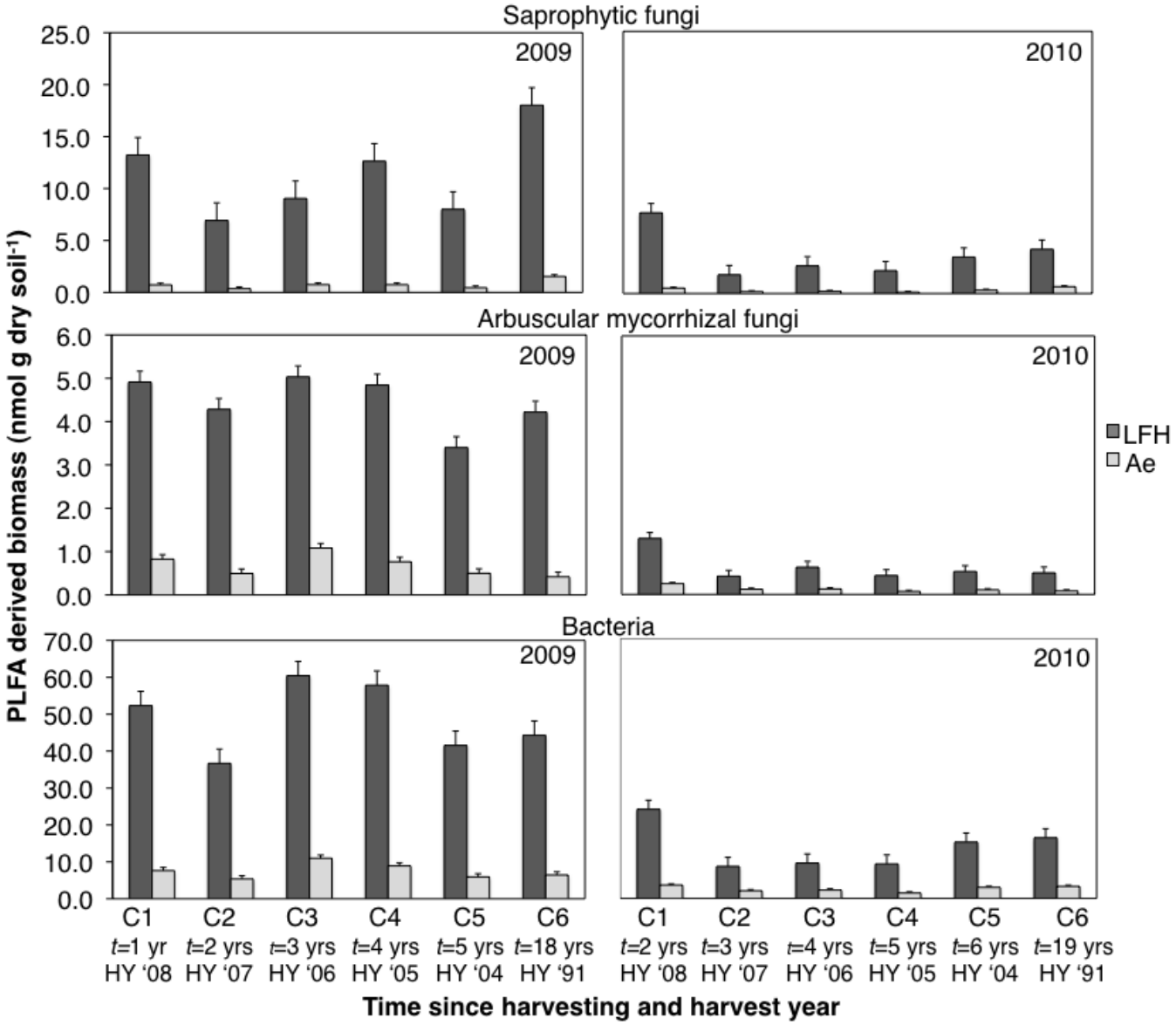

Figure 4.2. Mean (+/- standard error; *n*= 9) biomass of saprotrophic fungi (top), arbuscular mycorrhizal fungi (middle) and total bacteria (bottom) as indicated from phospholipid fatty acid biomarkers, in units of nmol/g lyophilized soil. Soils samples were from the LFH and Ae horizons of six cutblocks (C), each harvested in a different year (HY), ranging from 2008-1991, sampled in June of 2009 and 2010.

correlated with community composition with an r^2>0.8 (depicted as a vector) but in 2010 the correlation was weaker and thus not reported.

The ordination was able to incorporate 94% of the total variability of the dataset in 2009, and 95% of the total in 2010. Axis 2 in both sampling years represented soil horizon, indicating that 31% of the difference in the community composition was related to soil horizon in 2009, and 14% related to horizon in 2010. Again, as seen in the relationship with stand age, the trend was more distinct in 2009 samples.

4.5.4 Genetic biomarker derived bacterial community composition

The bacterial community in the most recently harvested cutblock clustered separately from the older cutblocks in both 2009 and 2010 (Figure 4.4), and the effect of stand age was significantly related to bacterial community composition (p<0.001). Dendrogram analysis also indicated a clear separation of bacterial populations based on cutblock age, corroborating the NMS ordination results. In the NMS ordinations, the age of cutblocks within the chronosequence generally separated out along axis 1 in both sampling years, which represented 26-29% of the total variation within the dataset. There was some grouping by soil horizon (p<0.05) in both NMS and the dendrogram analysis, but it was a weaker grouping variable compared to stand age. Soil horizon was not captured by the axes generated by the NMS ordination, therefore, must have represented < ~20 % of the variability in the community data. The A statistic indicated that there was more homogeneity within the 'age' and 'horizon' groups than expected by chance.

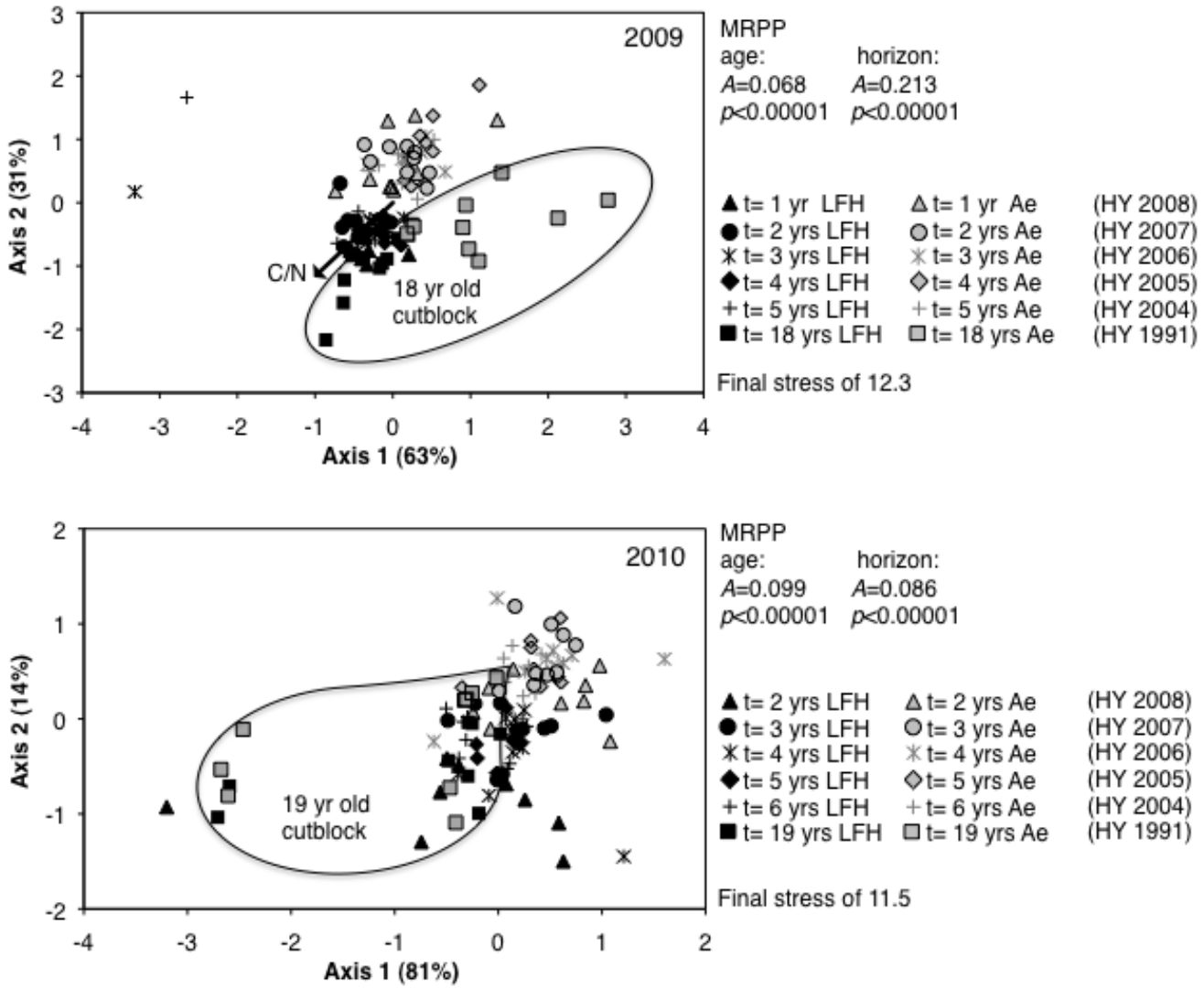

Figure 4.3. Non-metric multidimensional scaling and multiple response permutation procedure (MRPP) analysis of phospholipid fatty acid (PLFA) biomarkers from a chronosequence of six cutblocks sampled in 2009 (top) and 2010 (bottom). In 2009, cutblocks were 1 – 18 yrs post-harvest and in 2010 they were one year older; 2 – 19 yrs post-harvest. Vector represents Pearson correlation values of ≥0.8 with carbon/nitrogen (C/N) ratio in 2009 samples. Data was analyzed in log transformed, mol% format. The *A* statistic indicates within group homogeneity; an *A* value 1 means the samples within a group are identical, *A*=0 would indicate a level of homogeneity expected by chance, and an *A* of around 0.3 is typical for microbial datasets (McCune and Grace, 2002).

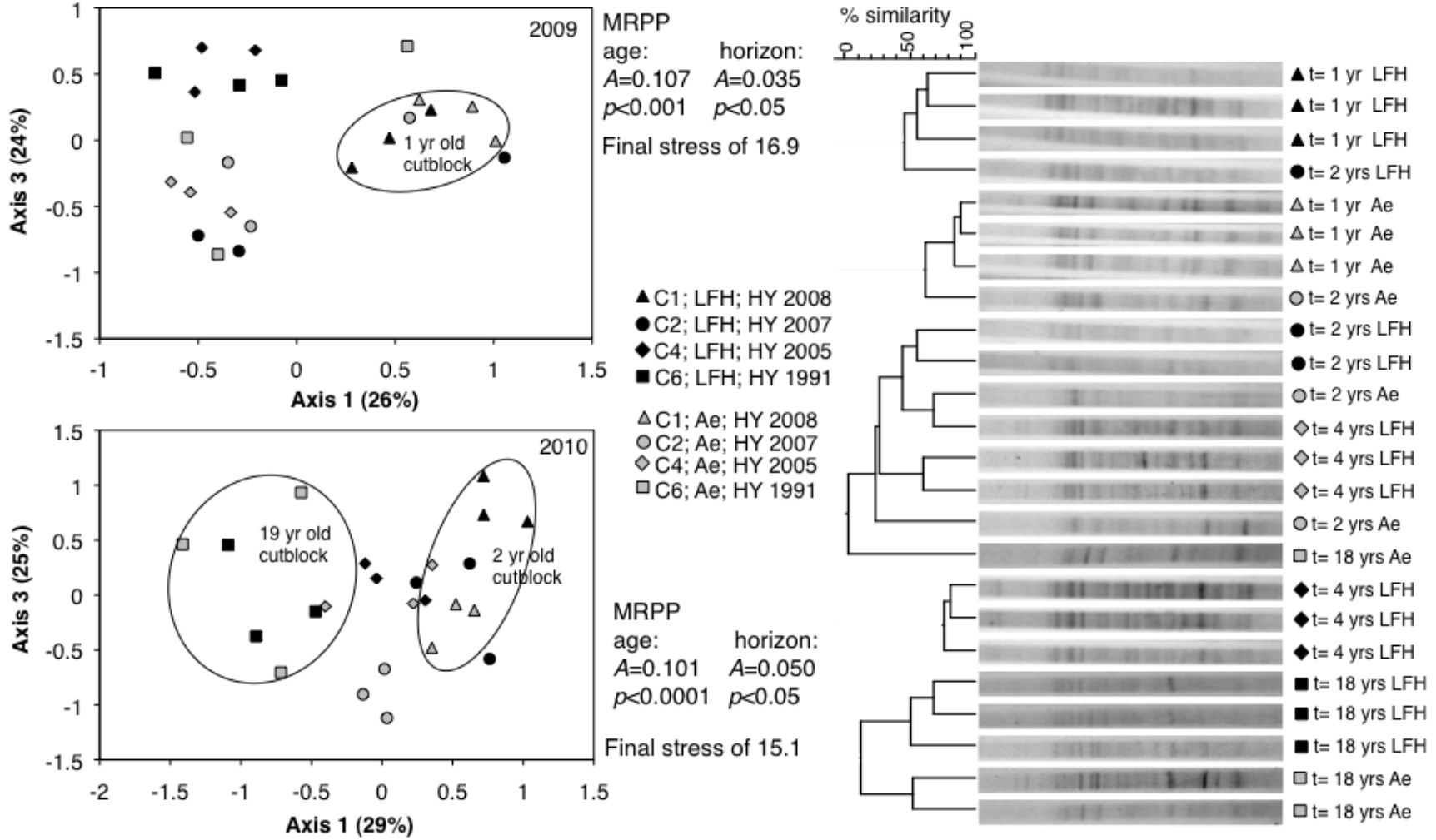

Figure 4.4. Non-metric multidimensional scaling and multiple response permutation procedure (MRPP) analysis (left) and dendrogram analysis (right) of 16S rDNA polymerase chain reaction denaturing gel gradient electrophoresis (PCR-DGGE) bands from clear-cut forest soils sampled in 2009 and 2010 at various ages post-harvest (dendrogram 2009 samples only). NMS and MRPP were analyzed by age and soil horizon on log transformed, mole % data. The A statistic indicates within group variance; an $A<0$ indicates less variation within a group than chance would expect, and an A=0.3 is typical of microbial datasets (McCune and Grace, 2002). Vectors represent Pearson correlation r values of $\geq$0.8 with soil water content (GWC) and organic carbon (C_{org}). Dendrogram analysis was based on Pearson correlations of DGGE banding patterns.

4.6 Discussion

4.6.1 Microbial biomass

4.6.1.1 Bacteria

The results of this study displayed the resilience of the bacterial community to changes in vegetation cover and microsite associated with post-harvest succession. The biomass, that is, the size of the bacterial community as indicated by PLFA biomarkers, did not differ between recently cut (1 – 6 yrs post-harvest) and ~20 yr old sites (Figure 4.2) in the chronosequence. Chapter 3 of this thesis corroborated the resilience of bacteria to harvesting disturbance in which a cutblock was sampled pre- and post-harvest in a time series. Here too, the bacterial biomass did not decrease from pre- to post-harvest microsite conditions. Similarly, other research addressing the impact of harvesting on microbial communities have found biomass to remain consistent despite the disturbance (Hannam et al., 2006; Pennanen et al., 1999).

A change in biomass after harvesting, or lack thereof, is a reflection of the changes that occurred in the soil microsite as repercussions of the removal of the trees, or subsequent erosion/leaching. In this study, the carbon and nitrogen content of the soil was not affected, the LFH was not entirely removed (Table 4.1), and the soil water content was more closely related to annual ppt fluctuations than to the effect of harvesting, so the vital needs of the microorganisms remained intact, sustaining their population size. As Hannam et al. (2006) also postulated, if done well, harvesting operations can be unobtrusive enough than the microbial community will not be detrimentally disturbed. In this case, sufficient water, substrate and nutrients remained on site, irrespective of vegetation removal and succession, such that the same microbial population size was sustained.

What did stand out in this study as a strong influence of microbial biomass was soil moisture content, which was a reflection of spring ppt, measured over the two months previous to sampling in each year. In 2010 soil samples, the bacterial biomass was significantly lower than it had been in 2009 (Figure 4.2), while the soil moisture content was twice that of 2009 moisture levels (Figure 4.1). During the spring of 2010, 71.2 mm more rainfall fell than during the summer of 2009 (May 1 – June 30 2009 total: 82.1 mm; May 1 – June 30 2010 total: 153.3 mm), and the soil water content consequently doubled (Table 3.1).

Soil moisture has long been known to be a strong influence on the general soil microsite and especially on microbial activities (Brady and Weil, 2002; Moore-Kucera and Dick, 2008). These results illustrated another example of the importance of soil moisture on microbial populations. In this case, the impact of a doubling in soil moisture appeared to be greater than the impact of harvesting. Analysis of correlations between soil moisture and chemical soil parameters (Chapter 3) supports this theory in that soil carbon (both total and organic), nitrogen, C/N ratio, pH, N, P and K availability, and microbial biomass were all correlated with soil moisture at a $p<0.05$ or lower, and this was consistent in both horizons. Substrate availability (C_{total} and C/N ratio) did not differ between 2009 and 2010 sample years (Table 4.1), indicating that carbon source can be discounted as a potential reason for the decrease in biomass in 2010. Thus, I hypothesize that the June 2010 soil samples were saturated with water to the point that conditions had become oxygen limited (relative to the soil in June of 2009). The oxygen depletion may have limiting enough to have caused microbial biomass to decline. Furthermore, the soil moisture content seems to have had a greater impact on microbial biomass than the harvesting disturbance. Moore-Kucera and Dick (2008) concluded similarly from their study of

the impacts of harvesting that moisture, temperature and C inputs related to seasonal changes were more influential on the microbial community than harvesting itself.

4.6.1.2 Saprophytic fungi

In contrast to bacterial biomass, the fungal biomass (inferred from PLFA biomarker 18:2ω6) differed significantly along the chronosequence. In 2009, there was significantly more fungal biomass in the ~20 yr old site than in the first 5 yrs post harvest (Figure 4.2). Contrastingly, the opposite trend was observed in 2010 where the largest fungal biomass occurred in the youngest tree stand as opposed to the oldest. In either case, the fungal biomass responded more acutely to harvesting than bacterial biomass in these results as well as in Chapter 3 of this thesis, and elsewhere (Bååth, 1980; Bååth et al., 1995; Hassett and Zak, 2005; Lundgren, 1982; Pennanen et al., 1999).

The larger amount of total fungal biomass in the ~20 yr old site relative to the younger ones (in 2009) was likely because of the difference in the rooting systems of early successional vs. older tree stands. An older tree stand may support a more populous fungal community because there would be a greater availability of living mature woody biomass, which provide fungi with necessary lignified substrates (Hassett and Zak, 2005) than younger, less woody successional plant communities could provide. Additionally, the larger rooting systems of mature trees could support greater ectomycorrhizal communities which live in symbiosis with tree roots, and have been found to represent the majority of the fungal community of coniferous forest soils (Finlay and Soderstrom, 1989). The root systems of younger cutblocks, though they may remain in the soil, are dead and cannot sustain symbionts like ectomycorrhizae long after the stem has been cut (Harvey et al., 1980).

It has been suggested that there is a 'lag time' after harvesting in which the fungal community thrives on decomposing tree roots (Moore-Kucera and Dick, 2008), and the results of Chapter 3 have potentially exhibited this delayed response as well with regards to total microbial biomass. The current results depicted this 'lag time' in the 2009 data, where the youngest sites yielded the second highest total fungal biomass, and in the 2010 data where the youngest site actually surpassed the biomass of the ~20 yr old stand. The response of the fungal biomass to clear cutting exhibited a lag-effect in which the youngest cutblock had larger fungal biomass than intermediate cutblocks. As seen in the 2009 data (Figure 4.2), the oldest cutblock supported the largest fungal community due to the presence and interactions with mature trees.

As described above, wetter conditions in 2010 was the postulated explanation for why the fungal biomass was starkly lower in 2010. Again, the effect of soil moisture seemed to overshadow any effect of harvesting because the trend of biomass by cutblock age was altered in 2010. It is reasonable to suggest that moisture content exceeded harvesting effects in young cutblock especially, because microsite conditions like temperature and moisture are knowingly more extreme in freshly-cut stands (Moore-Kucera and Dick, 2008) because they do not have mature trees to buffer the soil system from changing environmental conditions.

4.6.2 Microbial community composition

Both methods of assessing microbial community composition determined that the community composition was different in recently cut vs. ~20 yr old cutblocks. There are many factors that may contribute to the shift in community over the ~20 yrs of succession after clear cutting, and most are consequential of the change in plant cover. The loss of mature coniferous trees alters the deposition of organic material on the surface. As successional vegetation such as

grass, aspen and willow trees colonize, fresh organic matter will be deposited, but substrate quality and chemistry will differ from pre-harvest characteristics (Myers et al., 2001). The death of mature tree roots will also result in a change of the quantity and quality of root exudates (Moore-Kucera and Dick, 2008). The roots themselves, which form as hosts for mycorrhizal infection and a source of decomposable material, will also differ between successional plants vs. mature trees, potentially selecting for different microbial groups and shifting the community structure (Grigal, 2000). With harvesting comes a loss of understory plants as well as the mature trees, and this has been shown to decrease the plant diversity overall in post-harvest replanted forests (Hernesmaa et al., 2005; Shaw et al., 2004). This loss in plant diversity may further the selectivity of the microsite conditions for different microorganisms compared to those that inhabited the mature forest soils. Wetter (Figure 4.1) (Zak et al., 1999), warmer (Zogg et al., 1997), and more extreme variations of the microsite of recently cut stands may also play a role in shifting the community composition away from that of a more mature tree stand. Unfortunately, an old growth tree stand was not available for this chronosequence study so a direct comparison cannot be made between the chronosequence sites and an old growth site, but Moore-Kucera and Dick (2008) found that community composition was still different than an old growth forest 8 yrs after clear cutting, but by 25 yrs the community had returned to a likeness with the old growth forest soils.

The community composition also differed in each of the soil horizons measured in this study, and this was likely due to the significant differences in microsite conditions of each horizon (Table 4.1) such as substrate quality (Myers et al., 2001), moisture (Zak et al., 1999), or pH (Bååth et al., 1995), all of which were significantly different between LFH and Ae soils.

4.6.2.1 Comparing the results of PLFA vs. DNA fingerprinting

Community differences between young (1 – 5 yrs) and older (~20 yrs) sites post-harvest and between soil horizons were detected by both PLFA and 16S rDNA fingerprinting techniques, and both lead to the same general interpretation that the community differed between young and older cutblocks. Phospholipid fatty acid analysis suggested a more drastic differentiation between the communities in each horizon than DNA fingerprinting did. The oldest cutblock in the chronosequence was the most distinctly different via PLFA analysis, and the younger sites were relatively more similar to each other. Contrastingly, DNA fingerprinting analysis indicated that the youngest cutblock in the chronosequence was the most distinctly different. Non-metric multidimensional scaling analysis indicated that stand age accounted for around 80% of the total variation in the PLFA dataset from 2010, whereas in the DNA dataset from 2010 that number was only 29%. The relationship with stand age was more strongly inferred from PLFA data, as indicated by 80% accountancy of variability rather than 29%. This was inherent in the fact that PLFA is a community level analysis, whereas 16S rDNA was species specific and is thus more complicated to group in an ordination. Community composition shifts due to harvesting were captured by both PLFA and molecular techniques, and the overall interpretations were the same for either method in a general sense. However, ordination of PLFA data captured markedly more of the total variation within the data set than DNA ordination. For ecosystem level disturbances studies, PLFA analysis provides a more efficient method of quantifying and qualifying microbial responses. In harvested soils of AB, changes in the bacterial population assessed by DNA fingerprinting paralleled the response of the entire microbial community as a whole.

4.7 Conclusions

This chronosequence study illustrated that total soil microbial biomass did not change over the years post-harvest, nor did the bacterial biomass. Fungal biomass, which is known to be more sensitive to disturbance than bacterial biomass, was largest in the oldest tree stand in the chronosequence. The composition of the microbial community changed along the post-harvest chronosequence. Ultimately, these results implied that changes in microsite conditions associated with post-harvest ecosystem succession induced more pronounced changes in the composition of the community than the size of the community (Pennanen et al., 1999). Wide variation between precipitation in the spring months of 2009 and 2010 suggested that soil moisture had a greater influence on microbial biomass than harvesting did. Though significant differences in the microbial community composition of soils in a post-harvest chronosequence were found, future work is needed to explore the connection between microbial community composition and function, in relation to soil productivity.

5.0 RELATIONSHIP BETWEEN AMMONIA OXIDIZING BACTERIA AND BIOAVAILABLE NITROGEN IN HARVESTED FOREST SOILS OF CENTRAL ALBERTA

5.1 Preface

Chapter 3 illustrated the direct, short term impact of harvesting on community level microbial communities, while Chapter 4 more specifically examined the bacterial community's response to harvesting using molecular techniques. In both chapters, it was determined that microbial biomass remained unchanged by harvesting, but that the community composition was altered. Chapter 5 continues to build upon the investigation of the microbial community composition, assessing a specific functional group of bacteria called the ammonia oxidizers. These bacteria catalyze the rate determining step in the N cycle of forest soils, which are N limited in the first place and, therefore, are of importance in forestry. The objective of this study was to assess the changes in ammonia oxidizing bacterial community composition in response to harvesting, and link the community response to availability of inorganic N forms NH_4^+ and nitrate NO_3^-. To address this research objective, a subset of the chronosequence used in Chapter 4 was used in conjunction with N bioavailability data. I hypothesized that there would not be discernable differences in the community composition along the chronosequence, nor would there be a relationship with N bioavailability. I did, however, expect to see differences in community composition between the LFH and Ae horizons.

5.2 Abstract

Forest soils are commonly limited in nitrogen (N), and the removal of above ground biomass in harvesting operations can exacerbate the problem. Thus, the soil organisms that facilitate the rate-limiting step in the N cycle, the oxidation of ammonium (NH_4^+), are of special interest in harvested environments. The objective of this study was to investigate the changes in ammonia oxidizing bacteria (AOB) that occurred in the years following clear cutting, and link those community shifts to availability of inorganic N forms NH_4^+ and nitrate NO_3^-. Genetic fingerprinting targeting the *amo*A gene coupled with denaturing gel gradient electrophoresis was carried out over two summers on forest floor (LFH) and mineral (Ae) soils of three cutblocks harvested during different years but similar in every other respect. *In situ* NO_3^- and NH_4^+ were measured over the growing seasons of 2009 and 2010, as well as a suite of soil characteristics. Results indicated that the AOB community composition differed in younger vs. older cutblocks, but not by soil horizon. The changes seen in the AOB paralleled the change in N bioavailability across sites, soil horizons, and sampling years, thus indicating that N bioavailability may be directly linked to AOB community composition. This link may provide the basis for the use of AOB as indicators of nutrient availability in the future.

5.3 Introduction

Forest ecosystems are commonly limited in nitrogen (N) and the transformation of N from one form to the next is a tightly coupled cycle (Rennenberg et al., 2009). Nitrogen limitation is exacerbated in harvested soils, where not only has the N in the biomass itself been removed, but subsequent leaching, erosion, and increased N cycling rates deplete nutrient availability further (Chanasyk et al., 2003; Grigal, 2000). The rate-determining step in forest N cycles is often the oxidation of ammonia or ammonium (NH_4^+) to nitrite (Laverman et al., 2001), which is catalyzed by ammonia oxidizing bacteria (AOB) and archaea.

Chemolithotrophic AOB are aerobic, obligate autotrophs (Wrage et al., 2001) that use NH_4^+ as their sole source electron acceptor for respiration and carbon dioxide as their chief source of carbon (Kowalchuk and Stephen, 2003). They are responsible for the rate limiting step in nitrification in a wide variety of environments, thus they are crucial in the global N cycle (Kowalchuk et al., 1997). All AOB are categorized within the evolutionary lineage called *Proteobacteria*. Also known as purple bacteria, the *Proteobacteria* are separated into 5 subdivisions, two of which, β and γ, have AOB lineages. Within the β subclass, which is the terrestrial group and thus the relevant organisms in this study, there are two genera of ammonia oxidizers referred to as *Nitrosomonas* and *Nitrosospira* (Bäckman et al., 2004; Laverman et al., 2001). Both of these genera can be further subdivided into clusters that respond differently to environmental conditions such as pH, salinity, acidity and NH_4^+ availability (Kowalchuk et al., 2000; Kowalchuk et al., 1997; Laverman et al., 2001). Cluster groups 1, 2, and 4 of the *Nitrosospira* lineage are dominant in acidic forest soils with low NH_4^+ availability (Mintie et al., 2003), while cluster 3A have been identified in disturbed soils with high quantities of NH_4^+

(Boyle-Yarwood et al., 2008; Kowalchuk et al., 2000; Laverman et al., 2001; Mintie et al., 2003; Yeager et al., 2005).

Studies of forest soil AOB have identified a number of environmental factors that may influence AOB community composition. For example, in the only published study concerning AOB in harvested soils, Bäckman et al. (2004) found that AOB community composition shifts due to clear cutting. They concluded that the change in AOB community structure was driven by NH_4^+ availability and nitrification potential associated with harvesting disturbance. Irrespective of clear cutting disturbances, other forest soil studies have linked AOB community shifts to such environmental factors as NH_4^+ content (Hastings et al., 1997; Mintie et al., 2003), nitrification potential (Bäckman et al., 2004; Yeager et al., 2005), vegetation cover (Boyle-Yarwood et al., 2008; Nugroho et al., 2005), temperature (Avrahami and Conrad, 2005), changes in pH (Bäckman et al., 2003; Kowalchuk et al., 1997; Yeager et al., 2005), and C/N ratio (Nugroho et al., 2005). Contrastingly, Avrahami et al. (2002) concluded that AOB community composition did not respond to varying NH_4^+ concentrations and Laverman et al. (2005) found there was no relationship between AOB and nitrate (NO_3^-) or NH_4^+ production rates.

Molecular techniques offer a means of investigating AOB community composition. Polymerase chain reaction (PCR) coupled with denaturing gel gradient electrophoresis (DGGE) is one means of assessing community composition and does so by separating unique genetic sequences in a polyacrylamide gel based on nucleotide composition (Muyzer et al., 1993; Nicolaisen and Ramsing, 2002). In 1997, Rotthauwe and others developed a primer set to target a 491 bp fragment of the ammonia monooxygenase subunit A gene (*amoA)*, thus enabling researchers to investigate bacteria responsible for NH_4^+ oxidation by targeting the enzyme that catalyzes the reaction, ammonia monooxygenase. This gene is present in all AOB, and is

believed to contain enough information to make phylogenetic inferences based on its sequence (Nicolaisen and Ramsing, 2002; Rotthauwe et al., 1997).

Nitrogen limitations represent a challenge for the long-term productivity of managed forests and the response of AOB, the organisms catalyzing the rate limiting step in the N cycle, are scarcely understood. Increasing understanding of this functional group of bacteria may help characterize and delineate N deficiencies in the environment (Yeager et al., 2005). With this in mind, the objective of this study was to investigate the impact of clear cutting on AOB community composition using a chronosequential approach of three similar cutblocks of different ages on the Boreal plain of Alberta. The secondary objective was to identify whether N bioavailability could be linked with shifts in AOB community composition in these disturbed soils.

Soil from the forest floor (LFH) and the top 5 cm of the mineral horizon (Ae) were analyzed to account for the zones a) most rich in organic matter and bioavailable nutrients (Neville et al., 2002), and b) the most densely concentrated rooting zone (Strong and La Roi, 1985). Ammonia oxidizing bacteria community composition was determined using PCR-DGGE of the *amoA* primer set (Rotthauwe et al., 1997). To draw connections between changes in AOB and changes in N bioavailability, *in situ* NO_3^- and NH_4^+ was measured, as were general soil characteristics. The response of AOB community composition, bioavailability of N, and general soil characteristics were assessed at three differently aged cutblocks of the Boreal Plain, Alberta.

5.4 Materials and Methods

5.4.1 Site description

The study site was located in central Alberta (54°03' N, 115°84' W; 782 masl) on the Boreal Plain, 220 km northwest of Edmonton, Alberta. The region is underlain by Cretaceous and Tertiary sandstones, shales, clays and gravels ranging from <15 to >150 m thick (Pawlowski and Fenton, 1995). The predominant soil type is Orthic Gray Luvisol, which typically exhibits an organic LFH layer, an Ae horizon leached of clay, and a Bt horizon enriched in clay from the above Ae horizon (Agriculture Canada Expert Committee on Soil Survey 1987). These Luvisols are relatively rich in phosphorus (P) and calcium (Ca) because of high apatite and Ca content of the parent material (Smith et al., 2003). The predominant trees in the area are lodgepole pine (*Pinus contorta*), though jack pine (*Pinus banksiana*), white spruce (*Picea glauca*), black spruce (*Picea mariana*), balsam poplar (*Populus balsamifera*) and trembling aspen (*Populus tremuloides*) are also found in the area (Smith et al., 2003). The region experiences mean summer and winter temperatures of around 10 °C and -15 °C, respectively, and an average of 500 – 600 mm of precipitation annually (Environmment Canada, 2000; Strong and Leggat, 1992).

Three cutblocks were established as sampling sites based on consistent soil type (Orthic Gray Luvisol) and vegetation cover of >80% lodgepole pine before harvesting. One cutblock was harvested in 2007 (HY 2007), the second in 2005 (HY 2005), and the third in 1991 (HY 1991). All three cutblocks were clear-cut in January (woody debris left onsite) to minimize soil compaction and the two more recently harvested sites (HY 2005 and HY 2007) were treated with glyphosate herbicide (Vision, Monsanto Canada Inc.) at a rate of six liters per hectare during the first growing season after harvesting.

5.4.2 Soil sampling methodology

Cutblocks were divided into a systematic sampling plan and sampled in June 2009 and 2010. Each cutblock was separated into 3 transects, each transect with three sample plots, and each sample plot a composite of four randomly chosen subsamples. Soil was collected using a JMC Backsaver probe (Clements Assoc. Inc, Newton, IA) with a 3.2-cm diameter tip. Samples were gathered from the LFH and Ae at each sample location for a total of 54 samples per year (i.e., 3 plots x 3 transects x 2 horizons x 3 cutblocks = 54 samples per year). Transects were set up along the slope gradient (if any) to account for any differences associated with slope position and spread across the entire cutblock to account for as much inter-site variability as possible. Soils were stored on ice until returning to the lab, at which point they were sieved with a <2 mm mesh and stored at 4 °C. One set of subsamples was air-dried and pulverization for general soil characterization, and a second set was stored at -80 °C for subsequent DNA extraction and amplification.

5.4.3 Characterization of soil properties

All soil analysis were carried out based on the Soil Sampling and Methods of Analysis Book (Canadian Society of Soil Science, 2008). Gravimetric water content (GWC) was determined by calculating the mass lost after drying a known quantity of soil at 105 °C for 48 h, and expressed as mass of water per unit mass of dry soil (%). Soil pH was measured in a 2:1 soil: double deionized water slurry. Total C (C_{total}) and N (N_{total}) were quantified by the combustion method using a LECO CNS-2000 and expressed as a percentage (%) of the total soil mass

(Helgason et al., 2009). The carbon to nitrogen (C/N) ratio was calculated by dividing the C_{total} by the N_{total} and thus reported here unit-less.

5.4.4 *In situ* bioavailable nutrient measurements

In situ NO_3^- and NH_4^+ fluxes were measured using Plant Root Simulator (PRS^{TM}) Probes (Western Ag Innovations, Saskatoon, SK). This technology consists of an ion exchange membrane that emulates the nutrient sorption and surface characteristics of plant roots, thus assesses potential nutrient supply rates. In other words, the flux of nutrient into the PRS^{TM} probe supplies a surrogate measurement of plant available nutrient concentration in soil water, hereafter referred to as bioavailable N.

Probes were installed on the same date, and in the same location as soil samples for a total of 54 separate measurements per summer. A 6-week burial time was utilized for the PRS^{TM} probes to ensure saturation would not occur (Western Ag Innovation, PRS^{TM} probe Operations Manual), thus probes were replaced mid-July of each summer with a fresh probe in the exact same "soil slot" for a total burial time of 12 weeks. Upon removal, probes were cleaned with deionized water immediately and stored at 4 °C for subsequent analyses by Western Ag Inc.

Nutrients were eluted from the PRS^{TM} probes with 0.5M HCl. A segmented flow Autoanalyzer III (Bran and Luebbe, Inc., Buffalo, NY) was used to quantify N and P colourimetrically. Phosphorus and Ca were quantified by a PerkinElmer Optima 2000-DV inductively coupled plasma mass spectrometer (PerkinElmer Inc., Shelton, CT).

5.4.5 Analysis of ammonia oxidizing bacteria (AOB)

DNA extraction

Total DNA was extracted from 0.5 g of LFH and Ae soil using the FastDNA Spin Kit for Soil (MP Biomedicals, Montreal QC). Cell lysis was performed by vigorous shaking using a bead beater according to manufacturer's instructions. DNA extract quality was evaluated by electrophoresis on a 1% agarose gel stained with CybrSafe according to manufacturer's instructions (Invitrogen, Burlington ON) and visualized on a Bio-Rad Gel Doc XR System with Image Lab Software (Bio-Rad, Mississauga, ON).

Amplification of amoA gene

From the DNA extract, the *amoA* gene was amplified using primer set *amoA* 1F-gc (5'-CCG CCG CGC GGC GGG CGG GGC GGG GGC ACG GGG_GGG GTT TCT ACT GGT GGT-3') and 2R (5'-CCC CTC KGS AAA GCC TTC TTC-3') (Rotthauwe et al., 1997). This gene represents the functional group within the β–*Proteobacteria* that are capable of oxidizing ammonia. The optimized PCR reaction recipe consisted of 2 μL 1:50 diluted DNA extract; 0.5 μM of primers 1F-gc and 2R; 10 μL HotStarTaq Plus Master Mix (Qiagen, Mississauga, ON) 0.6 mM $MgCl_2$; 0.125 μg BSA (Amersham Biosciences); and 6.88 μL ddH_2O to make a total reaction volume of 20 μL. PCR was carried out with an initial 5 min incubation at 95 °C, followed by 39 cycles of 45 sec denaturing at 95 °C, 45 sec annealing at 58°C and 45 sec extension at 72°C. The final step holds at 95 °C for 15 sec, 60 °C for 30 sec, and again 95 °C for 15 sec (Helgason et al., 2010).

PCR product was confirmed on a 1% agarose gel stained with CybrSafe according to manufacturer's instructions (Invitrogen, Burlington ON) and visualized on a Bio-Rad Gel Doc XR System with Image Lab Software (Bio-Rad, Mississauga, ON). The product was then

concentrated overnight at -20 °C with 0.1× total volume of 3M sodium acetate and 2.5× total volume of 70% ethanol to remove superfluous PCR ingredients. Concentrated DNA was re-suspended in TE buffer and final DNA concentration determined using a NanoDrop2000 Spectrophotometer (Thermo Scientific, Ottawa ON) and corresponding software.

Denaturing Gel Gradient Electrophoresis

Denaturing Gel Gradient Electrophoresis was then performed on combined *amoA* PCR product using a Bio-Rad DCode system (Bio-Rad, Mississauga, ON). Approximately 600 ng of DNA from each sample was loaded onto an 8% polyacrylamide gel with a 35–65% denaturing gradient of formamide and urea. Electrophoresis was carried out for 16 hrs at 80 V and 60 °C. The resulting gels were stained with CybrSafe (Invitrogen, Burlington ON) in 1× TAE buffer for 0.5 hrs, de-stained for 0.5 hrs and photographed using a Bio-Rad Gel Doc XR System with Image Lab Software (Bio-Rad, Mississauga, ON). Using a sterile scalpel, random dominant bands were excised and eluted in H_2O for 45 min at 37 °C. Eluted DNA was re-amplified using the same primers and temperature program as above. Confirmation of re-amplified fragments was carried out on 2% agarose gel, followed by excision and purification with the GeneClean II Kit (Qbiogene, Carlsbad CA) by manufacturer's instructions. Finally, re-amplified DNA from the DGGE bands were sequenced by the Plant Biotechnology Institute (Natural Resources Canada, Saskatoon SK) and submitted to the GenBank database to confirm AOB origins (BLAST algorithm).

5.4.6 Statistical Analysis

Significance testing

Data exploration, correlations and significance testing for soil parameters was carried out with SPSS Version 17.0 for Windows (SPSS Inc., 2008). Log transformed soil parameters were tested for normality (Shapiro-Wilks) and homogeneity of variance (Levene's Test), and were determined to be non-normal and heteroscedastic. Thus the Kruskal Wallis test and Spearman's rank correlations, neither of which violate parametric assumptions, were used for significance testing (Field, 2005).

Analysis of DGGE gels

Band detection, gel analysis and cluster analysis of DGGE images were carried out in Bionumerics v.5.1 software (Applied Maths, Austin, TX) (Helgason et al., 2010; Ma et al., 2008). DGGE band identification was set for a least square filtering cut-off below 1.26% and background subtraction (disk size) of 12.3%. Band selection utilized a minimum profiling of 9.0%, a position tolerance of 0.5% and a 0% optimization. Based on densiometric curves and the Ward linkage method, cluster analysis was performed using the Pearson correlation coefficient. A binary presence-absence matrix was then created by band matching derived from the densiometric curves (Helgason et al., 2010; Peixoto et al., 2006). Species richness was inferred from the number of bands detected by Bionumerics software in each sample (Smith et al., 2010).

Ordination

Non-metric multidimensional scaling (NMS) analysis using PCOrd v.5.0 (MjM Software Gleneden Beach, OR) was used to draw significance from the DGGE presence-absence matrix (Helgason et al., 2010; Smith et al., 2008). Ordination was performed with the Sørenson distance measure in the Autopilot Slow and Thorough mode (McCune and Grace, 2002). The starting

configuration was optimized in previous ordinations to achieve the lowest stress. A Monte Carlo test of significance as well as a Multi-Response Permutation Procedure (MRPP) was subsequently used to test for differences between independent groups.

5.5 Results

This study explored AOB community composition in soils of three cutblocks, each harvested in a different year (HY 2007, HY 2005, and HY 1991, respectively), and sampled in June of 2009 and 2010 from the forest floor and Ae horizon. Ammonia oxidizing bacteria community composition, bioavailable NO_3^- and NH_4^+ and general soil characteristics were measured at all sites in both soil horizons and both sampling years. Soil water content (GWC), total C (C_{total}), total N (N_{total}), carbon to nitrogen (C/N) ratio, pH, bioavailable NO_3^- and NH_4^+ were measured as soil parameters because they are all likely to have an affect on microbial community composition.

5.5.1 Soil characteristics and bioavailable N

5.5.1.1 Effect of stand age

The C/N ratio as well as NO_3^- and NH_4^+ availability decreased along the chronosequence as stand age increased (p <0.01) (Table 5.1). Contrastingly, stand age was not associated with GWC, C_{total}, N_{total} or pH. The C/N ratio was between 23 – 36 in the 2 – 5 yr post-harvest LFH soils, and 15 – 21 in the 18 – 19 yr post-harvest soils (Table 5.2). Except for the HY 2005 cutblock samples taken in 2009, all three sites and both horizons exhibited a decrease in the C/N ratio as time since harvest increased. Bioavailability of NO_3^- and NH_4^+ both decreased continually as stand age increased (Figure 5.1), but again with the exception of the HY 2005 cutblock 2009 samples. The proportions of NO_3^- and NH_4^+ of the total N bioavailability were roughly equal, and there were generally equal amounts of NO_3^- and NH_4^+ available in both the LFH and Ae horizons. The effect of sampling year was not significant for either C/N ratio or NO_3^- and NH_4^+ availability.

Table 5.1. Kruskal-Wallis significance test of gravimetric water content (GWC), total carbon (C_{total}), total nitrogen (N_{total}), carbon/nitrogen (C/N) ratio, bioavailable NO_3^- and NH_4^+, and pH from the LFH and Ae of three cutblocks from central AB harvested in 2007, 2005 and 1991; each sampled in June 2009 and 2010.

	GWC	C_{total}	N_{total}	C/N	NO_3^-	NH_4^+	pH
	----------%----------				------ μg/10 cm^2/12 week burial time ------		
Age	ns	ns	ns	**	***	***	ns
Horizon	***	***	***	***	ns	ns	ns
Sample Year	**	ns	ns	ns	ns	ns	***

**, ***Significant at the 0.01 and 0.001 probability level, respectively

† ns, non-significant at the 0.01 probability level

‡ Bioavailability of NO_3^- and NH_4^+ were measured as a flux into plant simulator (PRS™) probes in units of μg of nutrient per 10 cm^2 area of the PRS resin membrane over 12 weeks burial time in the soil column

§ GWC, C_{total} and N_{total} , expressed as a %, represents the mass of a variable (e.g. water) as a percentage of the total soil sample mass

Table 5.2. Total carbon (C_{total}), total nitrogen (N_{total}), gravimetric soil water content (GWC) and pH from the LFH and Ae horizons of three cutblocks from central AB. The cutblocks were harvested in 2007, 2005 and 1991 and sampled in June of 2009 and 2010. The *t i*ndicates the age of each cutblock at the time of sampling. Standard deviations are in brackets (*n*=9).

	C_{total}	N_{total}	C/N	GWC	pH
	----------% ----------			-----% -----	
		LFH			
t=2 yrs HY 2007	17 (7.3)	0.65 (0.33)	28 (6.4)	72 (49)	5.9 (0.30)
t=3 yrs HY 2007	18 (5.9)	0.78 (0.22)	24 (5.2)	160 (74)	5.1 (0.80)
t=4 yrs HY 2005	18 (6.7)	0.51 (0.18)	37 (6.4)	53 (26)	5.5 (0.20)
t=5 yrs HY 2005	13 (4.8)	0.54 (0.15)	23 (5.5)	99 (24)	4.9 (0.20)
t=18 yrs HY 1991	11 (3.1)	0.51 (0.16)	21 (3.0)	30 (8.3)	6.2 (0.50)
t=19 yrs HY 1991	9.4 (3.2)	0.20 (0.10)	15 (4.0)	67 (10)	5.2 (0.50)
		Ae			
t=2 yrs HY 2007	1.9 (0.95)	0.22 (0.20)	8.1 (2.1)	34 (48)	6.1 (0.30)
t=3 yrs HY 2007	2.0 (1.8)	0.050 (0.05)	4.9 (1.8)	36 (16)	5.3 (0.60)
t=4 yrs HY 2005	1.4 (0.27)	0.12 (0.02)	11 (1.5)	7.6 (4.1)	6.3 (0.10)
t=5 yrs HY 2005	1.6 (0.50)	0.18 (0.16)	15 (8.4)	34 (4.9)	5.1 (0.30)
t=18 yrs HY 1991	1.5 (0.45)	0.15 (0.04)	9.5 (1.0)	6.8 (3.8)	6.4 (0.10)
t=19 yrs HY 1991	2.1 (0.5)	0.60 (0.10)	8.5 (1.5)	28 (4.6)	5.2 (0.60)

‡ Bioavailability of NO_3^- and NH_4^+ were measured as a flux into plant simulator (PRS^{TM}) probes in units of μg of nutrient per 10 cm^2 area of the PRS resin membrane over 12 weeks burial time in the soil column

§ GWC, C_{total} and N_{total} , expressed as a %, represents the mass of a variable (e.g. water) as a percentage of the total soil sample mass

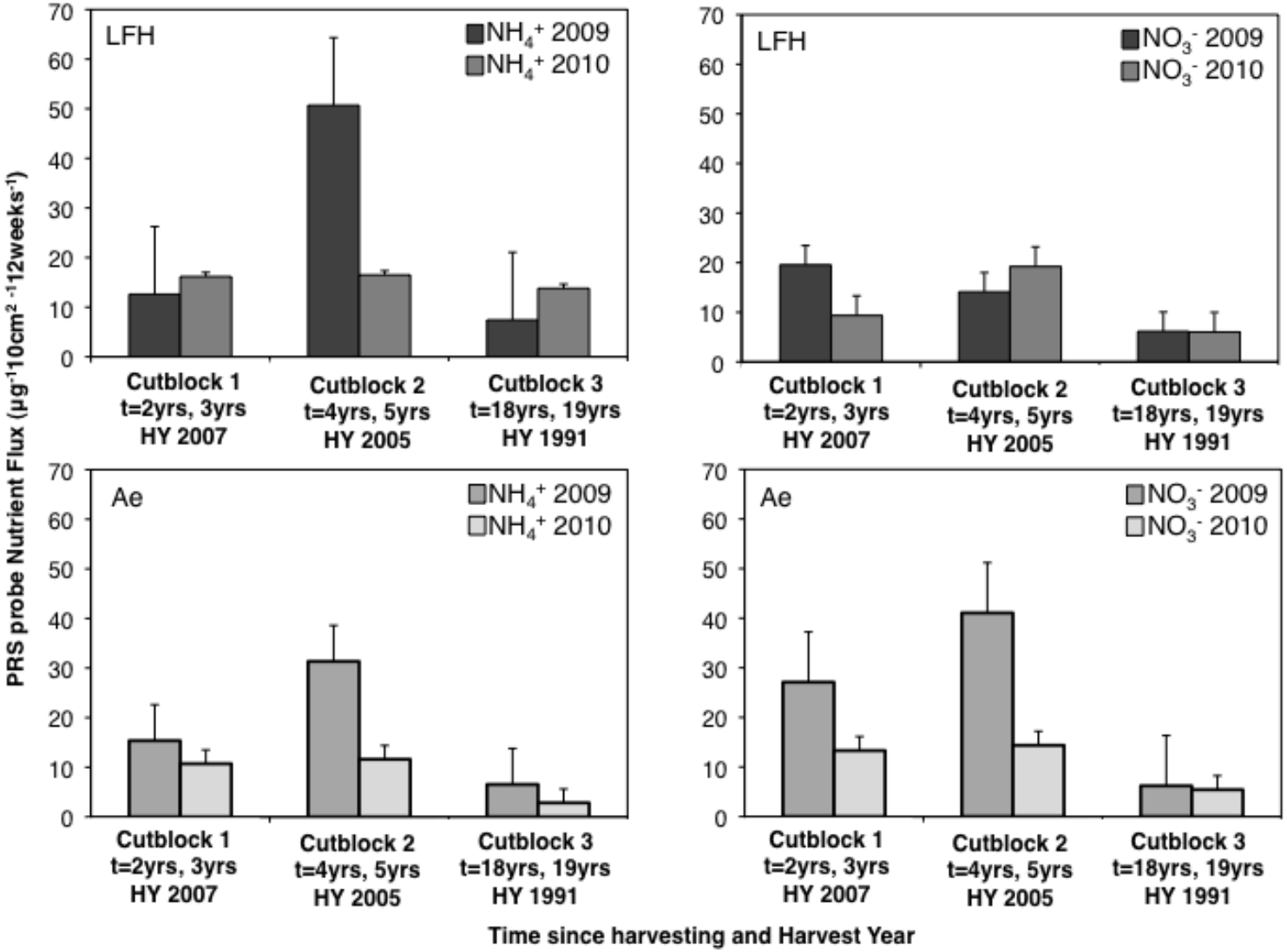

Figure 5.1. *In situ* bioavailable ammonium (NH_4^+) (left) and nitrate (NO_3^-) (right) measurements from cutblocks 1, 2, and 3 located in central AB which were harvested in January of 2007 (HY 2007), 2005 (HY 2005), and 1991 (HY 1991) respectively and sampled (*t* indicates the age of the cutblock at the time of sampling). Sampling took place over the 2009 and 2010 growing seasons in the LFH (top) and Ae (bottom) soil horizons. Error bars represent standard error (*n*=9).

5.5.1.2 Effect of soil horizon and sampling year

Soil water content, C_{total}, N_{total} and C/N ratio were all consistently lower in the Ae than the LFH ($p<0.001$), and these parameters were all correlated with each other at a $p<0.001$ (Table 5.3). The effect of sampling year was only significant on the soil water content and pH, and these two parameters were correlated with each other at a $p<0.01$. Between the two sampling years of 2009 and 2010, there was a large difference in the amount of spring precipitation measured over the two months preceding sampling. In the spring of 2010, there was 153.3 mm of rainfall, whereas only 82.1 mm of rain fell during that same period (May 1 – June 30) in 2009.

5.5.2 Ammonia oxidizing bacteria (AOB) community composition

The community structure of the AOB from these soils clustered by the independent variable of stand age, and both NMS (Figure 5.2) and dendrogram analysis (Figure 5.3) indicated this strong grouping by age. The dendrogram illustrated a general grouping of the younger samples at the top, and the older ones at the bottom. Multiple response permutation procedure significance testing supported this observation, indicating a highly significant effect of stand age on the AOB community composition ($p<0.0001$). Stand age was represented by axis 2 of the ordination, which accounted for 36% of the total variation within the dataset. The AOB community structure did not cluster by soil horizon, and MRPP analysis supported that the horizon effect was not a significant determinant of the AOB community.

Table 5.3. Spearman's rank correlations between gravimetric water content (GWC), total carbon (C_{total}), total nitrogen (N_{total}), carbon/nitrogen ratio (C/N), bioavailable NO_3^- and NH_4^+, and pH from three cutblocks from central AB harvested in 2007, 2005 and 1991; each sampled in June 2009 and 2010.

	GWC	C_{total}	N_{total}	C/N	NO_3^-	NH_4^+	pH
	----------%----------				-----μg/10cm^2/12 week burial time----		
GWC	1.0	0.553***	0.513***	0.510***	-.037	0.264***	-.251**
C_{total}		1.0	0.906***	0.831***	-.040	.268***	-158
N_{total}			1.0	0.584***	-.060	0.126	-.144
C/N				1.0	-.167	0.436***	-.111
NO_3^-					1.0	.252**	0.108
NH_4^+						1.0	-.122
pH							1.0

**, ***Significant at the 0.01 and 0.001 probability level, respectively

† ns, non-significant at the 0.01 probability level

‡ Bioavailability of NO_3^- and NH_4^+ were measured as a flux into plant simulator (PRS^{TM}) probes in units of μg of nutrient per 10 cm^2 area of the PRS resin membrane over 12 weeks burial time in the soil column

§ GWC, C_{total} and N_{total}, expressed as a %, represents the mass of a variable (e.g. water) as a percentage of the total soil sample mass

Upon sequencing of DGGE bands, it was determined that the AOB isolated in this study were uncultured bacterium from forested environments, with the exception of band #3 (Table 5.4) which was extracted from chloraminated water. Table 5.4 in conjunction with Figure 5.3 display the DGGE bands excised and sequenced, and the corresponding best-matches from the GenBank library (with their accession number).

The species richness of each sample was also inferred from the number of DGGE bands detected in each sample (Smith et al., 2010), and significance testing showed no difference between the species richness based on age, soil horizon, or sampling year, nor any significant correlations with soil chemical parameters (data not shown).

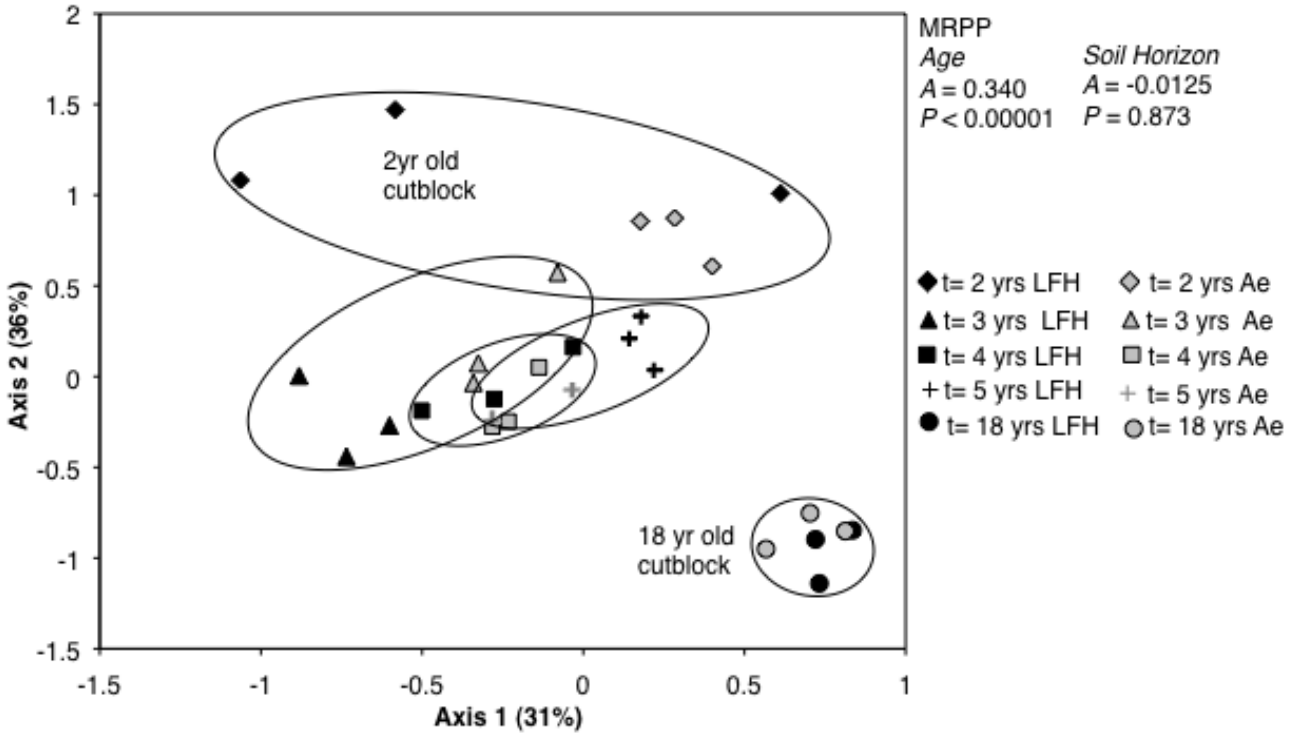

Figure 5.2. Non-metric multi dimensional scaling of *amo*A pcr-DGGE band patterns from the 2009 and 2010 sampling periods presented as a chronosequence by *t* as years after clear cutting. NMS analysis was carried out based on age and horizon with a final stress of 14.0. The *A* statistic indicates within group variance; an *A* value of 0.3 is typical for microbial datasets (McCune and Grace, 2002), and an $A<0$ indicates less variation within a group than expected by chance.

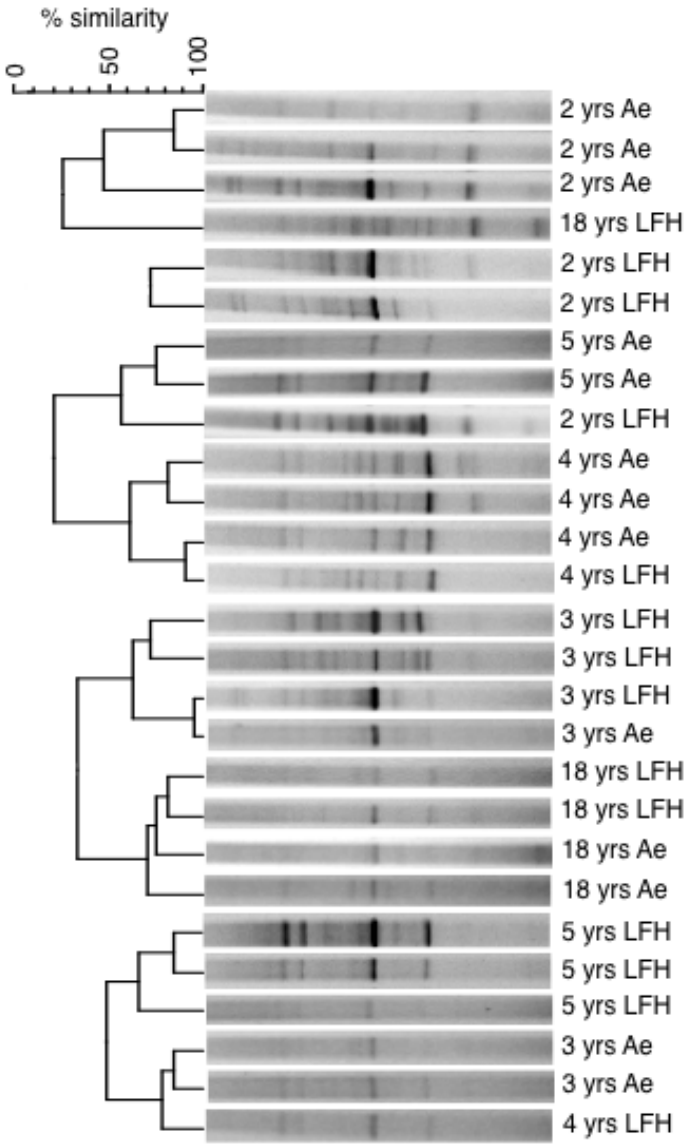

Figure 5.3. Dendrogram analysis of *amo*A pcr-DGGE band patterns from the 2009 and 2010 sampling periods presented as a chronosequence in years post-harvest. Clustering was carried out using Pearson correlation coefficients of the DGGE binary presence-absence matrix.

Table 5.4 Phylogenetic matches of sequenced DGGE bands from pcr-amplified *amo*A gene fragments. Sequences matched to GenBank library, using BLAST software.

DGGE band	Closest identity	Accession number	% similarity
1	Uncultured Nitrosospira sp. 14; ammonia monooxygenase subunit A	AY189141.1	98
2	Uncultured bacterium; partial amoA gene for ammonia monooxygenase subunit A, clone F-1065	AJ884520.1	100
3	Uncultured ammonia oxidizing bacterium; clone DW-ResA-10; ammonia monooxygenase gene	DQ181666.1	100
4	Uncultured Nitrosospira sp. 14; ammonia monooxygenase subunit A	AY189141.1	99
5	Uncultured bacterium; partial amoA gene for ammonia monooxygenase subunit A, clone A-286	AJ317884.1	98
6	Uncultured ammonia oxidizing beta proteobacterium HH-AOB-NP-23; ammonia monooxygenase subunit A	FN691162.1	97
7	Uncultured bacterium; partial amoA gene for ammonia monooxygenase subunit A, clone F-547	AJ317893.1	97
8	Uncultured bacterium; partial amoA gene for ammonia monooxygenase subunit A, clone C15A1	EF544025.1	98
9	Uncultured Nitrosospira sp. 14; ammonia monooxygenase subunit A	AY189141.1	98

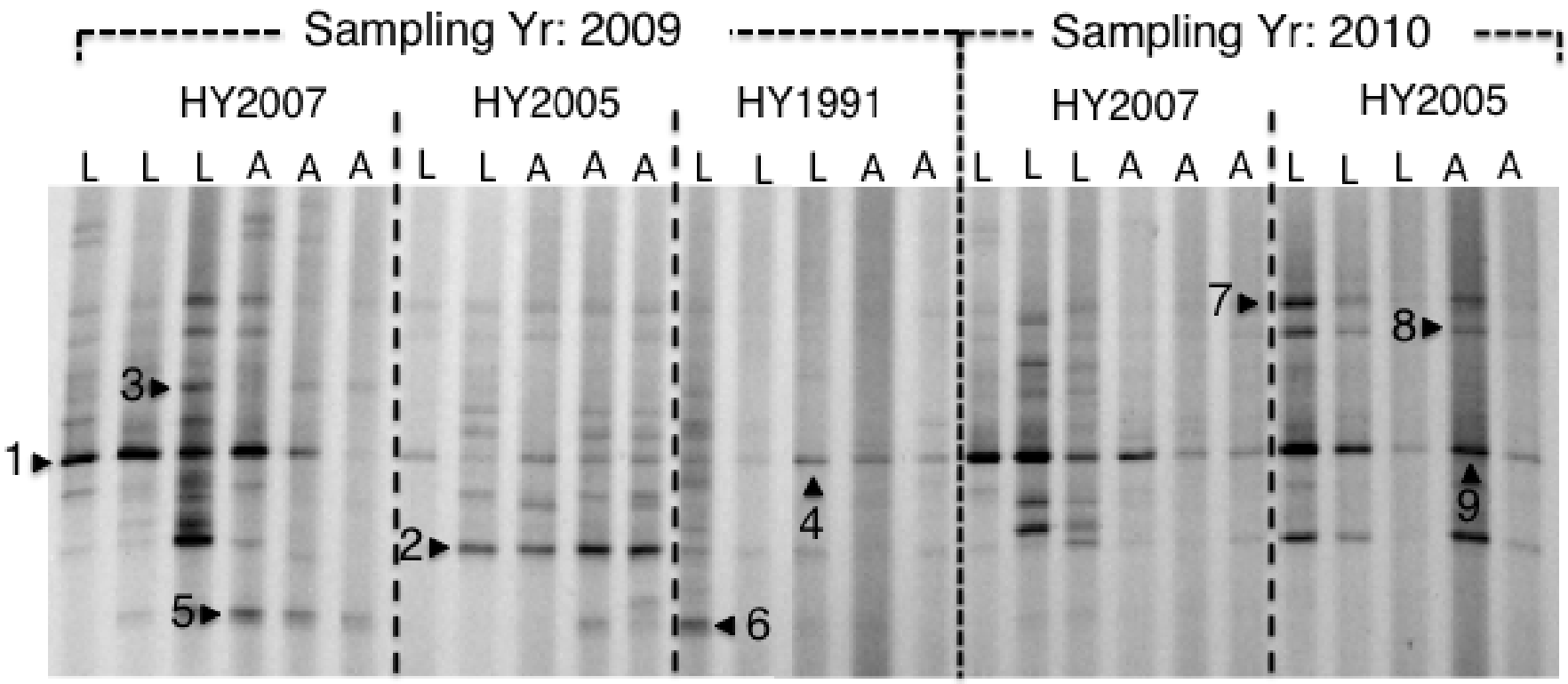

Figure 5.4. Denaturing gel gradient electrophoresis (DGGE) of PCR-amplified *amo*A gene fragments from three cutblocks located in central AB, which were harvested in January of 2007 (HY 2007), 2005 (HY 2005), and 1991 (HY 1991), respectively. Sampling took place over the 2009 and 2010 growing seasons in the LFH (L) and mineral Ae (M) soil horizons; each sampling location has two or three field replicates. Arrows and numerical labels represent sequenced bands whose closest identities are provided in Table 5.4.

5.6 Discussion

This section will focus first upon the change in AOB community composition and discuss the environmental factors, both the parameters measured in this study as well as those not measured here, that may have an influence on the AOB community. Secondly, an overview of the trends seen along the chronosequence of ~20 yrs post-harvest are described and explained.

5.6.1 Factors influencing AOB community composition

The results of this study indicated that the AOB community composition differed between recently harvested vs. older cutblocks. The y-axis of the NMS ordination (Figure 2) visually captures the succession of the AOB community accompanying the forest succession post-harvest. Early stage AOB may be of the *Nitrosospira* cluster 3A group who are adapted to relatively high NH_4^+ availability and respond favorably to disturbance (Yeager et al., 2005). As time passes, the community in concert with the above ground vegetation, shifts ecologically to a mid-successional stage that selects for AOB adapted for lower NH_4^+ availability and more stable microsites, which are likely members of clusters 1, 2 and 4 of *Nitrosospira* (Kowalchuk et al., 2000; Mintie et al., 2003; Yeager et al., 2005).

As in most microbial community studies, the difficult part is to elucidate which environmental parameters are actually driving the community structure to change. One means of identifying particular soil parameters that are linked to a community shift is to identify variables that responded similarly at both the site level and the horizon level as the AOB, representing two different scales of comparison. Of all the soil parameters

measured, NO_3^- and NH_4^+ availability were the only variables that changed in relation to stand age but not by soil horizon, which was the same response as the AOB community at the site and horizon scale. Thus, changes in N bioavailability in the years after harvesting paralleled the community shift of AOB post-harvest, suggesting that N bioavailability was related to AOB community composition in harvested soils.

This conclusion is supported by the study by Bäckman et al. (2004), which is the only other paper that has investigated the response of AOB to clear cutting. They too found that NH_4^+ availability was linked to AOB community composition and community size. They, as well as Nugroho et al. (2005), also identified a relationship between AOB community composition and nitrification rates. A study assessing AOB community changes in response to forest fire has also drawn connections between NH_4^+ availability and community composition, pointing to dominance of *Nitrosospira* cluster 3A in NH_4^+-abundant areas, and cluster 1, 2, and 4 dominance in later-succesional stage, undisturbed soils with lower NH_4^+ availability (Yeager et al., 2005). From these results, in concert with other studies of AOB communities, an understanding of the relationship between AOB and NH_4^+ and NO_3^- is emerging. Scaling-up from this study to the entire ecosystem at large, N availability may represent one of influential factors on AOB populations.

Recent literature has highlighted some other environmental variables that can drive AOB community composition. Vegetation cover has been identified as a community driver in studies by Nugroho et al. (2005) and Boyle-Yarwood et al. (2008), in which the contributions of plant species, through their organic matter inputs and influence on nitrification potentials have altered the community composition of AOB. The influence of vegetation cover may be especially relevant in the context of clear-cut

soils, since the plant structure and ecology is more drastically altered than in natural successional environments.

Another potential interaction that could have altered AOB community composition is the application of herbicides, commonly practiced in forest-managed areas. Two of the three cutblocks within this study (HY 2007 and HY 2005) were sprayed with glyphosate in the first growing season after clear cutting. Glyphosate has been demonstrated to shift microbial communities in soil (Lancaster et al., 2010; Mijangos et al., 2009), freshwater sediment (Widenfalk et al., 2008) and the rhizosphere of glyphosate resistant soybeans (Zobiole et al., 2010). Specifically relevant to the current study, Lancaster et al. (2010) found differences between the abundance of β-proteobacteria, which includes all known terrestrial AOB, at glyphosate treated *versus* untreated soils.

The influence of chemical soil characteristics on AOB community composition in this study was relatively minor. Changes in pH are often linked to AOB community structure (e.g., Nugroho et al., 2005) and different pH selects for certain AOB (Nicol et al., 2008), but since there was no significant difference in the pH at each site or in either soil horizon, it was not of great relevance to our research objectives.

5.6.2 Successional changes in the chemical soil system

5.6.2.1 Nitrogen

Availability of NO_3^- and NH_4^+ in these forest soils decreased over the successional years following clear-cutting. Bioavailable soil N loss after harvesting is commonly reported, so the decrease observed at these sites was expected. Loss of bioavailable N can be attributed to a combination of a few factors including: i) increased rates of erosion and

leaching of the nutrients from soils (Grigal, 2000); ii) a decrease in organic matter deposition associated with the initial removal of mature trees; and iii) following an initial and brief increase in mineralization and nitrification rates after harvesting (Walley et al., 1996), a longer term decrease in net mineralization and nitrification rates (Attiwill and Adams, 1993). Whether measured directly from the soil or indirectly from stream water nutrient concentrations, the general consensus is that bioavailability of N decreases as time since harvesting increases, corroborating the results of our study.

The soils of the cutblocks sampled here had roughly the same amount of NH_4^+ as NO_3^-, a slightly surprising finding for forest soils, which are often dominated by NH_4^+ due to tight N cycling and limitations (Attiwill, 1994). The fact that NO_3^- comprised a relatively large proportion of the total N pool implies that this system may not be N limited, and thus around half of the inorganic N is fully nitrified before being taken up by organisms, a transformation that would not occur in N limited environments. A second explanation could be that a large contingent of the organisms that take up inorganic N actually prefer NH_4^+ to NO_3^-. In fact, it has been shown that NH_4^+ is the preferred inorganic N form of many tree species (Glass et al., 2002; Rennenberg et al., 2009). In the case that NH_4^+ is actually the preferred inorganic N form of forest soil plants and organisms, the presence of NO_3^- in proportionally large quantities would be expected. In general, our understanding of N cycling and mechanisms of plant uptake are changing, and we still know relatively little about N cycling *in situ* as a whole (Schimel and Bennett, 2004). In the context of this study, the results indicate that this system is not cycling N as tightly as other N limited forest soils often do.

5.6.2.2 Carbon content

The C/N ratio of these soils decreased in relation to the time passed since harvesting. The C/N ratio of a soil often reflects the type of vegetation growing on it, so it is not surprising that the C/N ratio decreased in the successional years after harvesting as the ecology and age of the vegetation changes (Rennenberg et al., 2009). Woody, lignified mature trees have a high C/N ratio, which results in forest soils concurrently having high C/N ratios. Once those mature woody trees are removed and early successional grasses, shrubs, etc. with lower C/N ratios colonize the soil, the C/N ratio will soon reflect that of the vegetation (Binkley and Giardina, 1998). For example, grassland soils usually have a C/N ratio of ~10, whereas forest soil C/N is often $\leq$ 20 reflecting the relatively lower C/N of grasses than the woody biomass of trees (Brady and Weil, 2002). Organic inputs from successional vegetation in the form of both above- and below-ground organic matter will be more easily decomposable (relative to the woody substrate), bringing the C/N down over the years. Increased soil temperatures associated with the loss of over story shady vegetation can also increase decomposition and mineralization rates, thus further decreasing the C/N ratio over time (Moore-Kucera and Dick, 2008).

5.6.2.3 Moisture and pH

Neither the moisture content of the soils nor the pH changed in relation to time since harvesting in this study. The literature provides inconsistent results on whether clear cutting increases, decreases or is inconsequential for soil moisture content. Some, like the current study, find that moisture content does not change in relation to time since harvesting (Chang et al., 1995). The reason behind this lack of change is that the loss of

shady over story vegetation causes soil temperature to increase, which results in an increase in soil temperature and an ensuing increase in evaporation rates (Smith et al., 2010). Others, however, have found an increase in soil moisture in recently cut soils (Walley et al., 1996; Zhou and Sharik, 1997), attributed to a stark decline in evapotranspiration resulting from the removal of trees.

Soil pH did not change in relation to time since harvesting, and this lack of response is supported by other studies (Bååth et al., 1995; Simard et al., 2001; Smith et al., 2010). In fact, soil pH differed more between sampling years than it did over the entire chronosequence, indicating an interaction with roughly double the soil moisture from one year to the next. The resistance of the soil pH to change over the course of this ecosystem succession is often facilitated by soil Ca, which is the primary base cation contributing to cation exchange capacity (Alban, 1982). Because Ca concentration did not change in relation to stand age (Hynes and Germida, submitted), the resilience of pH to change over the years of succession is likely due to a buffering effect of Ca and was also observed by Simard et al. (2001). Forestry studies that focus on pH tend to compare the impact of wildfire with that of harvesting, and since fire has a greater impact on the soil ecosystem, changes to pH incurred by clear cutting are negligible in comparison. In any case, the results of this study indicate that soil pH did not change substantially over ~20 years of succession after clear-cutting in either the LFH or Ae horizon, and that changes in water content had a greater impact on soil pH than harvesting disturbance.

5.6.3 Ammonia oxidizer sequence matches

The sequence matches obtained from the GenBank library all shared at least 97% similarity with their respective AOB match, confirming the methodology used to target, amplify and separate AOBs using the 1F-gc 2R primer set (Rotthauwe et al., 1997) and DGGE separation technique. All sequences were matched with uncultured ammonia monooxygenase subunit A gene sequences, thus minimal information about cluster groups was gleaned from sequencing. The most dominant band that was present in all samples was matched with a *Nitrosospira* spp. isolated from a slightly acidic soil in the Cascade Mountains of Oregon (Mintie et al., 2003). All of the sequence matches, with the exception of one, were from samples of similar ecological context, namely organic and acidic forest soils, suggesting that the dominant AOBs sequenced in this study are common of forested ecosystems.

5.7 Conclusions

This is the first study to report the effect of clear cutting on ammonia oxidizing bacteria in the Boreal forest of North America. Our results were consistent with the findings of the only other study that has investigated AOB response to clear-cutting; that changes in NH_4^+ availability are likely related to shifts in AOB community composition after harvesting. Our results also indicated that the AOB community did not differ between the LFH and Ae in these disturbed sites. Regarding soil chemical parameters' response during the successional years post-harvest, bioavailability of N, in the form of NH_4^+ and NO_3^-, as well as the C/N ratios decreased over time since the disturbance. All of the other soil parameters tested including soil moisture content, pH, C_{total} and N_{total} did not change significantly over the ~20 yrs of succession measured in this chronosequence study.

Our research has captured the shift in AOB community composition occurring in concert with changing bioavailable N and vegetation cover associated with forest succession after clear cutting. Unfortunately, there still remains a disconnect between form and function in environmental microbiology studies, in that observing a change in community composition does not directly implicate a change in soil quality or site productivity. Some of the parameters measured in this study reflect changes associated with tree removal, namely a decrease in C/N ratio, increased N bioavailability and a shift in AOB community composition, but none of these changes indicate a decrease in site productivity. The fact that harvesting induces measurable changes to the soil ecosystem does not necessarily mean that those changes are detrimental, and that is the overarching conclusion of this study. The harvesting practiced utilized at these sites such as winter

harvesting and rake and burn site prep have successfully mitigated negative environmental impacts. This study supports that the forestry industry's best practices are a step in the right direction towards environmental stewardship and sustainable tree harvesting operations.

6.0 SUMMARY AND SYNTHESIS

Summary of research conducted

The research conducted in my M.Sc. thesis was in response to the need to increase understanding of how the soil ecosystem responds to harvesting disturbance on the Boreal Plain of Canada. In conjunction with parallel research on stream water, amphibians, vegetation, and hydrological responses to harvesting, a picture of forest management practices' role in the forest watershed at large is emerging. What made my research unique and novel was that I addressed the unknowns of how bacteria respond to harvesting using molecular tools, which has scarcely been studied. Genetic fingerprinting of bacteria using the universal bacteria gene as well as the functional group of ammonia oxidizers bacteria has shed light on bacterial response to harvesting at a phylogenetic level.

My study was separated into three research questions, each addressed in its own chapter and becoming more specific as each grew upon the last. First, I set out to find out how community level microbial composition and biomass was affected in the short term by clear cutting, as well as monitoring nutrient availability in the soil (Chap. 3). The answer, in general, was that microbial biomass did not decrease until two years after harvesting, and the reason for this remains unconfirmed. The two working hypothesis were that either there was a delayed response from the community, or that the doubling of moisture content two years post harvest was the cause of diminishing the microbial population. Community composition differed by cutblock age, and N, P, K, and Ca availability changed in predictable ways reported in other studies. Second, I assessed the microbial community size and composition over a chronosequence of ~20 yrs using both

community level and more specific phylogenetic fingerprinting of the bacterial community (Chap. 4). I found that biomass was resilient to change over the longer term, but community composition continued to diverge as stand age increased. The importance of soil moisture as an influence on microbial biomass was highlighted here too. Comparison of PLFA and 16S rDNA fingerprinting indicated that the same general story emerged from both methods of assessing microbial community composition. Most specifically (Chap. 5), I investigated the ammonia oxidizing bacteria using genetic fingerprinting in concert with N bioavailability at a subset of harvested cutblocks to determine their response to the disturbance and their relationship with soil N status. As with the community level and total bacterial population, the AOB community differed by stand age. The response of the AOB to harvesting paralleled that of N bioavailability at all sites, both sampling years and the two soil horizons, suggesting that there was a relationship between community composition and inorganic N availability.

Revisiting my hypothesis

Some of my hypothesis were confirmed throughout this study and thus accepted, while others were rejected. The hypothesis that microbial biomass would decline due to harvesting remains unconfirmed in regards to the short term. My interpretations of the decrease in biomass seen in 2010, two growing seasons after harvesting, were not conclusive as to whether the decline was due to a delayed response to harvesting, or a doubling in soil moisture that spring of 2010. In the longer term chronosequence study, however, my data suggest that my hypothesis was in fact wrong, and that moisture content had a greater impact on microbial biomass than harvesting.

A second hypothesis that was rejected in this study that regarding the AOB. I expected that there would not be discernable differences between community composition in cutblocks of various ages, nor would there be a relationship with N bioavailability. I was wrong in this expectation, and was surprised to find that the AOB community clearly differed in composition along the chronosequence, and shared a parallel response at the site and soil horizon level with N bioavailability.

Synthesis of trends and ideas

In summation of the results of each individual chapter of my thesis, I have concluded some of the trends that have emerged. The microbial community was generally resilient to harvesting disturbance. Throughout each chapter, the community composition changed in response to changing microsite conditions. Generally, though there still remains a question in regard to Chapter 3, the population size (microbial biomass) was more directly impacted by changes in moisture content than by harvesting. Both the microbial biomass resilience to harvesting and the relative dominance of moisture content as a biomass-determining factor have been reported in other studies. In all three chapters, each of which was assessed with a different fingerprinting method, the microbial community composition differed between cutblocks of varied ages. In Chapter 3, the comparison was between a 77 yr old tree stand and recently cut stands (1-2 yrs). The chronosequence study, Chapter 4, compared the community composition changes over ~20 yrs of succession. In Chapter 5, a subset of the chronosequence was used to compare AOB community composition changes. In all cases, whether assessed by PLFA (Chap. 3 and 4), 16S rDNA PCR-DGGE (Chap. 4), or *amo*A PCR-DGGE (Chap. 5), the

community composition of recently harvested stands was significantly different than the composition of older stands. Throughout each chapter, the LFH and Ae differed in absolute quantity of all parameters measured, but for all except Ca availability, the trends seen along the age chronosequence remained consistent in each horizon.

There are two major, overarching implications of this research. The impact of harvesting on the soil ecosystem was, generally, minor and this result was corroborated by other research (Hannam et al., 2006; Ponder and Tadros, 2002). The microbial biomass remained unchanged due to harvesting both in the short and longer term. Many of the soil parameters measured in these chapters were not disturbed either. Of the soil parameters that were altered by harvesting over the chronosequence, the changes were associated with improvements of soil productivity rather than detrimental effects. For example, the lowering of the C/N ratio over time or the slight neutralization of pH were both positive changes that can improve soil productivity. An idea that arose from these results, which is the second major implication of this research, was the notion that a change in community composition doesn't necessarily reflect a detriment to the ecosystem. Maybe instead, scientists need to shift their perception to view community shifts as an indicator of ecological resilience and adaptability. Of course there are scenarios where microbial biomass decreases and the community composition becomes less diverse in response to a disturbance, and this certainly would be a negative result. However, in this case, in which the biomass was not depleted and the community was changed rather than diminished, the alterations observed were not necessarily negative.

Limitations of the study

As in any study, this research project had some limitations. The most significant limitations of the study were in reference to the sampling design. The replicate (transects) design did not yield proper replicates, but instead pseudo replicates which were not entirely independent of one another. Rather than having three cutblocks that were harvested in 2008, for example, I had one cutblock harvested in 2008 with three transects erected along it. Another shortcoming was having taken a chronosequence approach to my sample design. The fundamental assumption of this type of study is that the differences seen between each site is a function of the time that has past since harvesting, rather than from inherent site differences. Though I tried to control for as many variables as possible among sites, this assumption was inevitably violated to some extent because the sites were not exactly the same.

My study carried some analytical limitations as well. Due to changes in staffing and expertise, my PLF data included in Chapter 4 was run on a different GC in 2009 than in 2010. Though I diligently and thoroughly assessed the appropriateness of comparing one GC's data to the next to correct for any discrepancies, it is acknowledged that differences between 2009 and 2010 microbial data could have been a relic of the differences between GC's to an extent. General PCR bias was also encountered in this study, as in any work that includes PCR. Any organism that comprises less than 5-10% of the total abundance of the community less likely to be amplified, therefore, not represented in the final DGGE fingerprinting analysis. There is nothing that I could have done to avoid this bias; it is inherent in PCR work. Analytical or otherwise, it is a

scientific responsibility to acknowledge a study's limitations, and make interpretations responsibly with those limitations in mind.

Future work

Future work would be complimentary and help delineate the entire story. As mentioned throughout my thesis, there still remains a gap in our understanding of the link between microbial community size and composition with their actual function, that is, productivity and activity, in the soil ecosystem. It would be extremely valuable to the environmental microbiological community in general, not to mention the FORWARD group, to develop a research program that could begin to make direct links between microbial community form and function. Only then will microbial communities be utilized as a direct indicator of ecosystem health. An example, which I have begun to investigate here, would be to measure AOB community shifts as a proxy for measuring N availability fluctuations in soils. On that same note, future research is needed to compliment the ammonia oxidizer component of this study. Ammonia oxidizing archaea (AOA) are recently discovered members of the nitrogen cycle, and have been found to be important in certain environments, oxidizing more ammonia than AOB in some cases. Since the focus of this study was more of a general characterization and a focus on bacteria, AOA were not investigated here, but to do so would be a valuable 'piece of the puzzle'. An assessment of AOA in relation to harvesting disturbance and N availability, along with a comparison with AOB would be a very interesting and useful M.Sc. project to contribute to the FORWARD project.

To conclude, my M.Sc. research has shown that microorganisms are generally resilient to and adaptable to the changes in microsite conditions associated with clear cutting. Harvesting practices carried out in central Alberta did cause measurable changes in the soil ecosystem, but in the 'big-picture' and building upon previous research, those changes were not necessarily detrimental nor long lasting. The most valuable lesson learned throughout this M.Sc with regards to my dataset is the notion that disturbance is not necessarily a 'bad word'. It is not synonymous with degradation. Another important concept gleaned from my dataset is this: just because a change is measurable, it does not mean that that change is large, or even ecologically relevant. I have certainly learned that lesson working with molecular techniques. Ecosystems have evolved to be adaptable and resilient, and will do just that in the face of environmental changes (within relatively minor magnitude and reasonable amount of time, of course). Even having spent two years studying the impact of anthropogenic disturbance on an ecosystem, I leave with a sense of hope that at least some anthropogenic disturbances are relatively minor, in the big picture, and are within the realm of adaptability of our forest ecosystems.

7.0 LITERATURE CITED

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APPENDIX A

Site description

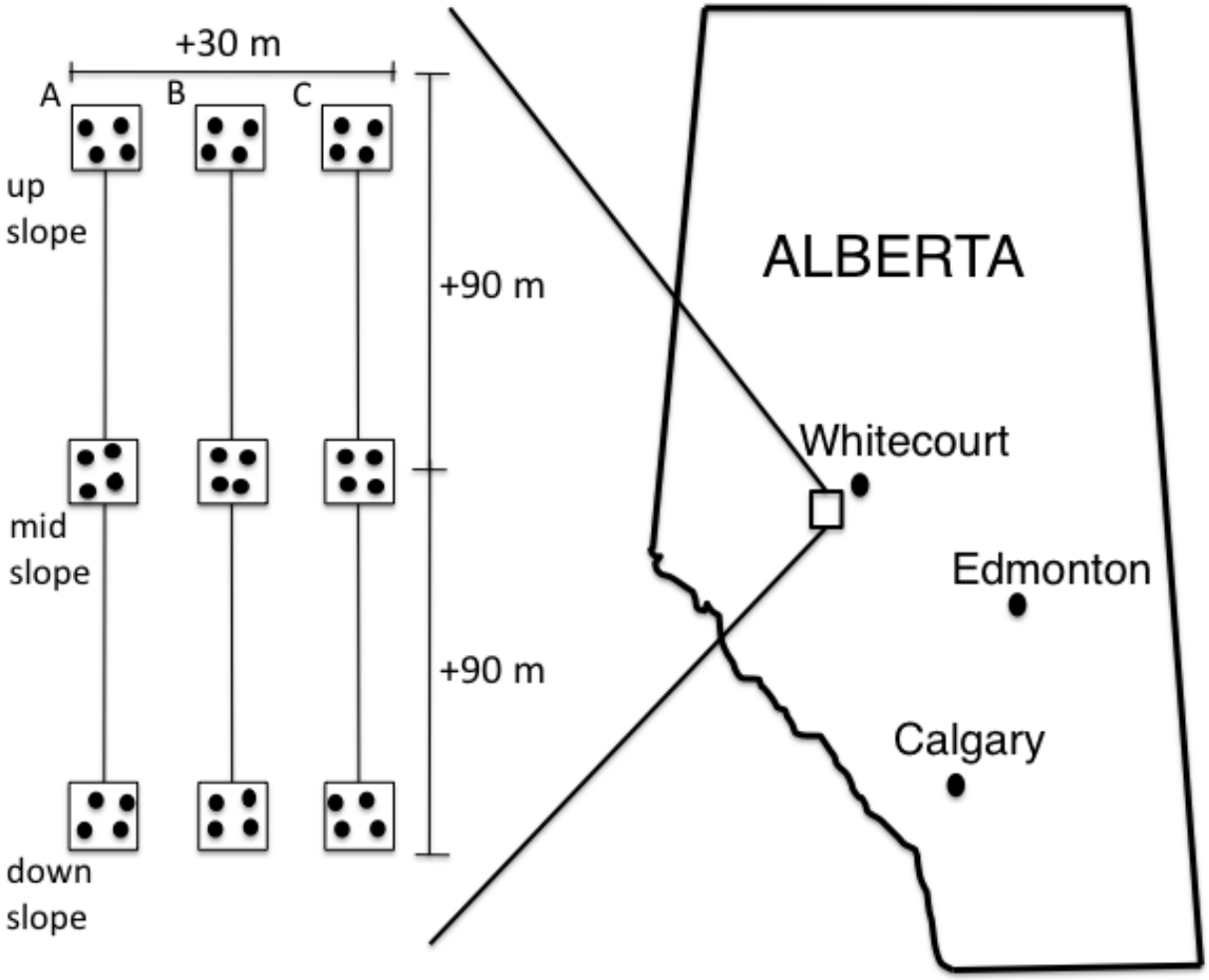

Figure A.1. Depiction of field site location and sampling design: three transects were created running across any slope gradient on each site. Each transect was at least 15 m away from the last, and sampling plots within a transect at least 90 m away from each other.

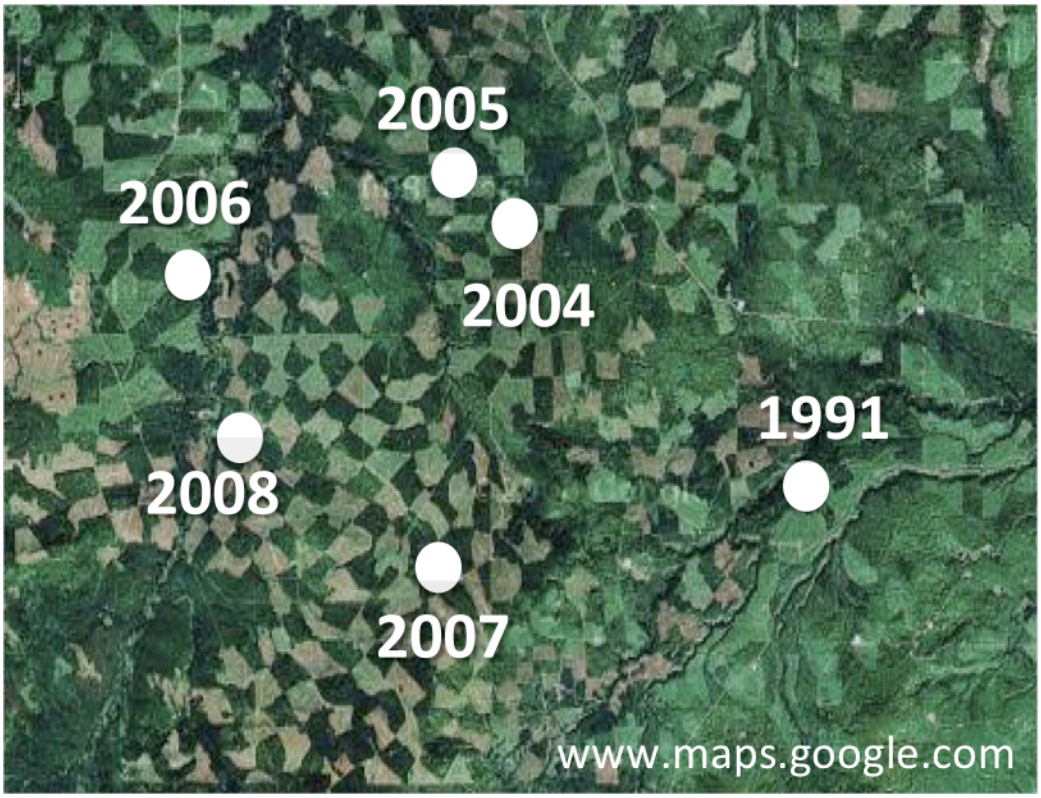

Figure A.2. Image from Google Maps of the cutblock area sampled in June 2009 and 2010 for soil, and PRS^{TM} probes installed at each site from June-August 2009 and 2010. The year indicates the time each cutblock was harvested.

Figure A.3. Photographs of each site in order of harvest year (HY) from 2008-1991 from top left to bottom right. Photos taken by H. Hynes, June 9th-11th, 2009.

APPENDIX B

Supplementary Nutrient bioavailability measurements

Table B.1. Full suite of bioavailable nutrients from PRS^{TM} probes installed from June-August 2009 in the LFH horizon of cutblocks harvested (HY) 2008-2004 and 1991. Nutrients measured were total inorganic nitrogen as the sum of ammonium (NH_4^+) and nitrate (NO_3^-) (N_{total}), NH_4^+, NO_3^-, calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), boron (B), sulfur (S), lead (Pb), aluminum (Al), and cadmium (Cd). All are reported as a flux into the PRS^{TM} probe in units of μg/10 cm^2/12 week burial time.

	HY 2008	HY 2007	HY 2006	HY 2005	HY 2004	HY 1991
N_{total}	1.2×10^2 (1.1×10^1)	32 (10)	33 (17)	65 (38)	39 (22)	14 (4.9)
NH_4^+	7.6 (5.0)	21 (9.2)	4.4 (1.8)	14 (20)	12 (16)	6.2 (4.4)
NO_3^-	1.2×10^2 (1.1×10^2)	13 (3.4)	29 (17)	51 (33)	27 (18)	7.4 (2.7)
Ca	1.9×10^3 (5.8×10^2)	4.2×10^3 (7.1×10^2)	3.0×10^3 (1.6×10^2)	1.9×10^3 (4.0×10^2)	2.2×10^3 (4.7×10^2)	3.2×10^3 (5.2×10^2)
Mg	3.6×10^2 (81)	4.9×10^2 (54)	5.7×10^2 (51)	3.4×10^2 (55)	4.0×10^2 (79)	5.0×10^2 (80)
K	7.5×10^2 (2.6×10^2)	1.6×10^2 (1.0×10^2)	3.0×10^2 (1.0×10^2)	3.8×10^2 (1.0×10^2)	3.7×10^2 (1.9×10^2)	3.4×10^2 (1.9×10^2)
P	68 (19)	36 (14)	49 (15)	29 (11)	54 (74)	15 (8.1)
Fe	62 (1.2×10^2)	71 (67)	2.5×10^2 (3.2×10^2)	65 (1.4×10^2)	53 (68)	27 (8.9)
Mn	1.6×10^2 (50)	1.1×10^2 (1.1×10^2)	1.6×10^2 (36)	2.1×10^2 (86)	1.6×10^2 (68)	10 (4.1)
Cu	0.10 (0.10)	0.30 (0.20)	0.30 (0.10)	0.20 (0.10)	0.10 (0.10)	0.20 (0.20)
Zn	7.9 (1.5)	7.8 (2.0)	11 (2.0)	8.3 (1.5)	8.3 (1.8)	2.7 (1.2)
B	2.3 (0.50)	3.3 (0.60)	3.1 (0.70)	2.0 (0.50)	2.2 (1.2)	3.5 (0.9)
S	1.1×10^2 (43)	1.5×10^2 (91)	98 (34)	75 (42)	76 (23)	61 (39)
Pb	0.30 (0.20)	0.30 (0.10)	1.3×10^2 (29)	0.30 (0.10)	0.40 (0.20)	0.10 (0.10)
Al	1.0×10^2 (38)	90 (26)	0.20 (0.20)	89 (17)	99 (19)	87 (13)
Cd	0.30 (0.10)	0.20 (0.10)	0.20 (0.10)	0.40 (0.20)	0.30 (0.10)	0.10 (0.10)

Table B.2. Full suite of bioavailable nutrients from PRS^{TM} probes installed from June-August 2009 in the mineral Ae horizon of cutblocks harvested (HY) 2008-2004 and 1991. Nutrients measured were total inorganic nitrogen as the sum of ammonium (NH_4^+) and nitrate (NO_3^-) (N_{total}), NH_4^+, NO_3^-, calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), boron (B), sulfur (S), lead (Pb), aluminum (Al), and cadmium (Cd). All are reported as a flux into the PRS^{TM} probe in units of μg/10 cm^2/12 week burial time.

	HY 2008	HY 2007	HY 2006	HY 2005	HY 2004	HY 1991
N_{total}	39 (20)	43 (19)	42 (33)	72 (85)	30 (29)	13 (2.4)
NH_4^+	16 (10)	27 (20)	21 (34)	41 (80)	6.8 (3.7)	6.2 (1.9)
NO_3^-	24 (14)	15 (5.4)	1.3×10^2 (11)	31 (31)	23 (30)	6.5 (1.4)
Ca	2.5×10^3 (5.8×10^2)	4.5×10^3 (6.0×10^2)	2.9×10^3 (7.4×10^2)	2.8×10^3 (6.9×10^2)	2.8×10^3 (3.6×10^2)	3.4×10^3 (3.3×10^2)
Mg	3.8×10^2 (78)	5.0×10^2 (67)	4.5×10^2 (1.4×10^2)	4.4×10^2 (55)	4.0×10^2 (42)	4.8×10^2 (52)
K	3.4×10^2 (1.2×10^2)	1.0×10^2 (83)	1.8×10^2 (1.6×10^2)	3.2×10^2 (1.6×10^2)	2.3×10^2 (1.4×10^2)	1.6×10^2 (20)
P	24 (10)	29 (17)	25 (7.5)	19 (6.3)	33 (38)	7.1 (2.8)
Fe	1.4×10^2 (82)	1.5×10^2 (1.4×10^2)	2.9×10^2 (2.2×10^2)	64 (27)	1.7×10^2 (1.5×10^2)	29 (5.9)
Mn	62 (32)	1.0×10^2 (67)	1.0×10^2 (44)	1.4×10^2 (65)	88 (34)	7.0 (2.8)
Cu	0.30 (0.20)	0.50 (0.40)	0.30 (0.10)	0.30 (0.10)	0.30 (0.10)	0.20 (0.20)
Zn	5.8 (1.4)	6.7 (2.5)	6.6 (2.7)	7.8 (1.6)	8.1 (2.8)	2.2 (0.7)
B	2.4 (0.90)	4.0 (0.80)	2.1 (0.80)	2.2 (0.30)	2.4 (0.70)	3.1 (0.40)
S	1.3×10^2 (47)	1.0×10^2 (43)	1.0×10^2 (41)	1.0×10^2 (51)	1.2×10^2 (38)	56 (28)
Pb	0.30 (0.10)	0.60 (0.40)	0.40 (0.20)	0.40 (0.20)	0.40 (0.20)	0.10 (0.10)
Al	1.8×10^2 (27)	1.0×10^2 (35)	1.4×10^2 (51)	1.5×10^2 (24)	1.5×10^2 (39)	1.0×10^2 (10)
Cd	0.20 (0.10)	0.20 (0.10)	0.20 (0.10)	0.20 (0.20)	0.20 (0.10)	0.10 (0.10)

Table B.3. Full suite of bioavailable nutrients from PRS^{TM} probes installed from June-August 2010 in the LFH horizon of cutblocks harvested (HY) 2008-2004 and 1991. Nutrients measured were total inorganic nitrogen as the sum of ammonium (NH_4^+) and nitrate (NO_3^-) (N_{total}), NH_4^+, NO_3^-, calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), boron (B), sulfur (S), lead (Pb), aluminum (Al), and cadmium (Cd). All are reported as a flux into the PRS^{TM} probe in units of μg/10 cm^2/12 week burial time.

	HY 2008	HY 2007	HY 2006	HY 2005	HY 2004	HY 1991
N_{total}	62 (33)	26 (4.2)	28 (10)	36 (20)	18 (4.0)	20 (24)
NH_4^+	5.5 (2.4)	9.4 (5.6)	7.1 (6.0)	19 (19)	3.4 (2.9)	6.0 (1.8)
NO_3^-	57 (32)	16 (4.8)	21 (8.7)	17 (8.4)	15 (2.3)	14 (24)
Ca	1.4×10^3 (5.3×10^2)	2.7×10^3 (1.1×10^3)	2.6×10^3 (7.0×10^2)	3.0×10^3 (2.3×10^2)	2.4×10^3 (3.8×10^2)	3.3×10^3 (5.5×10^2)
Mg	3.1×10^2 (88)	4.2×10^2 (84)	5.2×10^2 (56)	5.5×10^2 (37)	4.8×10^2 (65)	5.8×10^2 (95)
K	6.3×10^2 (1.8×10^2)	4.7×10^2 (1.3×10^2)	4.1×10^2 (1.1×10^2)	3.0×10^2 (1.3×10^2)	4.2×10^2 (1.6×10^2)	4.1×10^2 (91)
P	57 (14)	41 (13)	37 (12)	29 (18)	32 (14)	21 (7.4)
Fe	13 (4.3)	34 (77)	27 (18)	90 (1.2×10^2)	36 (26)	42 (82)
Mn	1.6×10^2 (55)	40 (27)	1.0×10^2 (42)	1.6×10^2 (69)	1.6×10^2 (25)	22 (36)
Cu	0.00 (0.00)	0.00 (0.00)	0.10 (0.10)	0.20 (0.20)	0.40 (0.80)	0.00 (0.00)
Zn	7.4 (1.7)	5.7 (1.4)	8.3 (1.8)	11 (2.9)	9.4 (1.5)	3.0 (1.9)
B	2.3 (0.70)	2.1 (0.50)	2.4 (0.40)	2.3 (0.40)	3.0 (1.5)	2.8 (0.7)
S	33 (11)	47 (25)	43 (26)	83 (67)	68 (25)	35 (24)
Pb	0.30 (0.10)	0.10 (0.10)	0.20 (0.10)	0.20 (0.20)	0.20 (0.10)	0.10 (0.20)
Al	84 (9.1)	73 (21)	89 (11)	1.1×10^2 (23)	1.1×10^2 (23)	99 (22)
Cd	0.40 (0.20)	0.30 (0.20)	0.50 (0.10)	0.40 (0.30)	0.50 (0.30)	0.20 (0.20)

Table B.4. Full suite of bioavailable nutrients from PRS^{TM} probes installed from June-August 2010 in the mineral Ae horizon of cutblocks harvested (HY) 2008-2004 and 1991. Nutrients measured were total inorganic nitrogen as the sum of ammonium (NH_4^+) and nitrate (NO_3^-) (N_{total}), NH_4^+, NO_3^-, calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), boron (B), sulfur (S), lead (Pb), aluminum (Al), and cadmium (Cd). All are reported as a flux into the PRS^{TM} probe in units of μg/10 cm^2/12 week burial time.

	HY 2008	HY 2007	HY 2006	HY 2005	HY 2004	HY 1991
N_{total}	39 (14)	24 (10)	27 (15)	26 (17)	17 (6.5)	8.2 (2.5)
NH_4^+	10 (7.8)	13 (8.7)	11 (9.6)	14 (16)	3.0 (3.1)	5.4 (2.6)
NO_3^-	29 (11)	11 (4.6)	17 (6.6)	12 (3.5)	14 (5.6)	2.8 (1.1)
Ca	1.5×10^3 (4.6×10^2)	3.7×10^3 (6.1×10^2)	2.3×10^3 (4.6×10^2)	2.9×10^3 (4.9×10^2)	2.3×10^3 (5.2×10^2)	3.0×10^3 (7.0×10^2)
Mg	2.8×10^2 (72)	4.3×10^2 (45)	4.4×10^2 (70)	4.7×10^2 (77)	3.9×10^2 (65)	4.8×10^2 (1.0×10^2)
K	4.9×10^2 (1.7×10^2)	1.9×10^2 (94)	3.6×10^2 (1.2×10^2)	3.2×10^2 (84)	3.2×10^2 (1.2×10^2)	1.9×10^2 (1.5×10^2)
P	28 (6.4)	18 (10)	25 (9.3)	21 (13)	18 (10)	7.4 (5.2)
Fe	98 (1.2×10^2)	1.1×10^2 (89)	1.7×10^2 (1.7×10^2)	1.5×10^2 (1.8×10^2)	1.1×10^2 (68)	15 (8.7)
Mn	65 (34)	56 (43)	94 (36)	1.3×10^2 (58)	1.1×10^2 (38)	7.0 (3.7)
Cu	0.00 (0.00)	0.40 (0.40)	0.20 (0.20)	0.30 (0.20)	0.20 (0.20)	0.20 (0.20)
Zn	5.6 (1.3)	6.3 (2.2)	7.2 (1.4)	9.1 (2.4)	7.5 (1.7)	2.2 (1.2)
B	1.8 (0.40)	2.4 (0.30)	2.1 (0.50)	2.6 (0.40)	2.7 (0.50)	2.2 (0.80)
S	70 (20)	92 (48)	63 (20)	1.2×10^2 (41)	96 (31)	28 (26)
Pb	0.20 (0.10)	0.40 (0.30)	0.40 (0.20)	0.40 (0.30)	0.30 (0.10)	0.20 (0.20)
Al	1.3×10^2 (20)	1.0×10^2 (30)	1.4×10^2 (31)	1.2×10^2 (18)	1.3×10^2 (27)	93 (36)
Cd	0.20 (0.10)	0.20 (0.20)	0.40 (0.20)	0.30 (0.20)	0.40 (0.20)	0.20 (0.10)